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## PUBLISHER'S ANNOUNCEMENT

With the present number Professor A. R. Cushny of University College, London, becomes joint editor with Professor John J. Abel of the Johns Hopkins University, Baltimore, of the JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS. At the same time, Sir T. Lauder Brunton of London, Professors J. T. Cash of Aberdeen, W. E. Dixon of Cambridge, J. A. Gunn of Oxford, Sir Thomas R. Fraser of Edinburgh, J. N. Langley of Cambridge, C. R. Marshall of the University of St. Andrews, R. Stockman of Glasgow, F. Ransom of London and Dr. H. H. Dale of London join the board of associate editors.

By this arrangement the ablest representatives of pharmacology in Great Britain unite with their American and Canadian colleagues in the conduct of the JOURNAL and the publishers feel confident that it will henceforth serve as the medium of publication for the best pharmacological researches of the English speaking countries.

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# THE PULMONARY ACTION OF VANADIUM TOGETHER WITH A STUDY OF THE PERIPHERAL REACTIONS TO THE METAL

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In a recent article<sup>1</sup> I have discussed briefly a few of the pharmacological actions of the metal vanadium. In the present instance I wish to describe some further observations which have been recently made with reference to the same subject.

## THE PULMONARY CIRCULATION

In the investigation of this subject I have made use of the methods generally employed for this purpose. The animals (dogs) were etherized and placed on the operating table. Sometimes the cervical cord was sectioned in order to avoid disturbing central influences. Arrangements were then made for recording the general arterial pressure from the right carotid artery and the thorax was opened by a median incision. To avoid hemorrhage the internal mammary artery and vein on each side were doubly ligated and divided between the ligatures. The section was made about one or two inches from the origin of the arteries. After opening the chest a perfectly regular artificial respiration was maintained throughout the remainder of the experiment. The animal's temperature was kept up by means of electric bulbs and the air and ether vapor were warmed before entering the lungs.

<sup>1</sup> Journal of Pharmacology and Experimental Therapeutics, 1912, iii, May, no. 5, pp. 477 to 515.

*Note:* A fairly complete bibliography of the literature dealing with the pharmacology of vanadium has been given at the end of my former paper. Consequently but few references need be given here.

The chest was usually opened a little to the left of the anterior mediastinum and the heart and right pulmonary sac were crowded over toward the right. By means of four stout ligatures inserted into the edges of the wound by which the thorax was opened the sides of the chest were pulled widely apart and tied in that position. The left pulmonary artery was then dissected out at the point where it passes out to the left from below the aortic arch. At this point the artery lies above the left bronchus and is covered over with a rather dense layer of pleura. The vessel is about 5 to 8 mm. in diameter in dogs weighing from 8 to 12 kg. If the pleural layer be gently dissected away it is an easy matter to pass an aneurism needle under the artery, lift it up and place two ligatures around it. The ligatures should be tied loosely at first and the arterial trunk should be isolated from the surrounding structures for a distance of about one-half to three-quarters of an inch. This is about the total available length of the arterial trunk. Beyond this (to the left) the vessel divides and passes into the tissues of the lung. The walls of the artery are much more easily ruptured than are those of systemic veins such as the femoral. This latter feature is often the cause of trouble when a sharp-edged serresfin or haemostat is placed on the artery preparatory to inserting the cannula. Only round-edged, very weak-sprunged serresfins should be used for this purpose, for a stiff-sprunged serresfin may break down the walls of the vessel at the angles where they are abruptly bent by the instrument. And a sharp-edged serresfin will almost surely wear a hole in the artery from the constant movement of the heart during the interval elapsing between the clamping off of the vessel and the adjustment of the cannula for recording the pulmonary pressure. I have found it convenient to use a special form of cannula for the pulmonary artery. This cannula is made of glass in the ordinary fashion but the point instead of being straight is bent at an angle of about 90 degrees to one side. When the point of the cannula is inserted into the artery and points directly toward the heart then the straight limb of the cannula points toward the head of the animal and is connected with the mercury manometer while the side (washout) tube of the cannula points ventrally out of

the chest and inclines a little over toward the right side of the animal. The opening in the point of the cannula should be about 3 or 4 mm. in diameter. When the tubes are filled (with sodium citrate solution) it is important not to leave more than 20 or 25 mm. of mercury pressure in the tubes for otherwise the solution may be forced directly into the heart when the serresfin is removed and the organ may thereby be greatly injured or completely stopped.

The solutions of vanadium which I have used in these experiments were made from sodium orthovanadate and were generally of a strength of 1 per cent. The vanadium salts were purchased either from Kahlbaum or from Eimer and Amend. Usually I have found it desirable to add a very small amount of hydrochloric acid to the solutions of the pure salts which are faintly alkaline in reaction. The neutralization of the solutions with hydrochloric acid is of considerable advantage in the course of the experiments for the animal may thereby be enabled to withstand the action of a larger quantity of the substance than would be the case with the slightly alkaline solution, although the specific action of the drug and the regular character of the records are not in anyway changed by a neutralization of the solutions.

Generally I have injected the drug into the femoral vein of the animal. Usually, also, as a sort of check on the accuracy of the experiment, I have recorded the systemic blood-pressure from the right carotid artery. And sometimes I have further recorded the volume changes of some of the abdominal organs such as the kidney. In all instances I have attempted to check up the results of the vanadium injections by the administration of epinephrine, barium, ergotoxine or some other substance whose action is well known.

By the method which I have used the recorded pulmonary pressure is concerned only with the right lung and the heart since the pulmonary artery going to the left lung is wholly occluded by the cannula. It would probably be possible to dissect out and use a branch of the left pulmonary artery (e.g., the division going to the upper lobe of the left lung) but I have not considered it necessary to do this.

Under the conditions above described I have found that the injection of 10 to 20 mg. of sodium orthovanadate causes a slow but prolonged rise in the pulmonary blood-pressure. This rise amounts to from 2 to 6 or 7 mm. of mercury. It begins in a few seconds after the injection but the maximum height may not be attained for two or three minutes or possibly even longer. The decline of the pressure again to the normal is also a slow process lasting usually somewhat longer than the time required for the rise. The elevation in the systemic pressure is usually rather rapid and the return to the normal may occur within one or two minutes. It is sometimes, however, prolonged over a considerably greater period than this. It would appear that the rise of pressure in the pulmonary artery is due to a number of factors. Among these may be mentioned, first, the possibility of an increase in the strength of the heart beat. A number of investigators have described a stimulating action of the substance upon the heart muscle. This has, in fact, been compared to digitalis. Personally I have never seen any very marked cardiac stimulation by the substance, but on the other hand I have often observed a weakening action on the heart even at the first injection of the drug. Consequently I am not inclined to believe that a stimulation of the heart itself should be held accountable for much of the increased tension in the pulmonary arteries. If the heart should be in no wise influenced directly by the substance, but should remain in its normal condition, I believe that there are still a number of other factors which are fully able within themselves to account for the pressure changes in the lungs. Second the extreme vaso-constriction which is produced in the abdominal organs by the metal is undoubtedly largely concerned in the increased pulmonary pressure. It seems that the left ventricle has to contract against this marked peripheral resistance with the result that there is a damming back of blood in the left auricle and pulmonary veins. This view is further upheld by the close correlation which exists both as to time and extent, between the increased pulmonary vascular tension and the peripheral constriction in the abdominal organs. Incidentally it may be mentioned that this same factor is undoubtedly mainly concerned in the

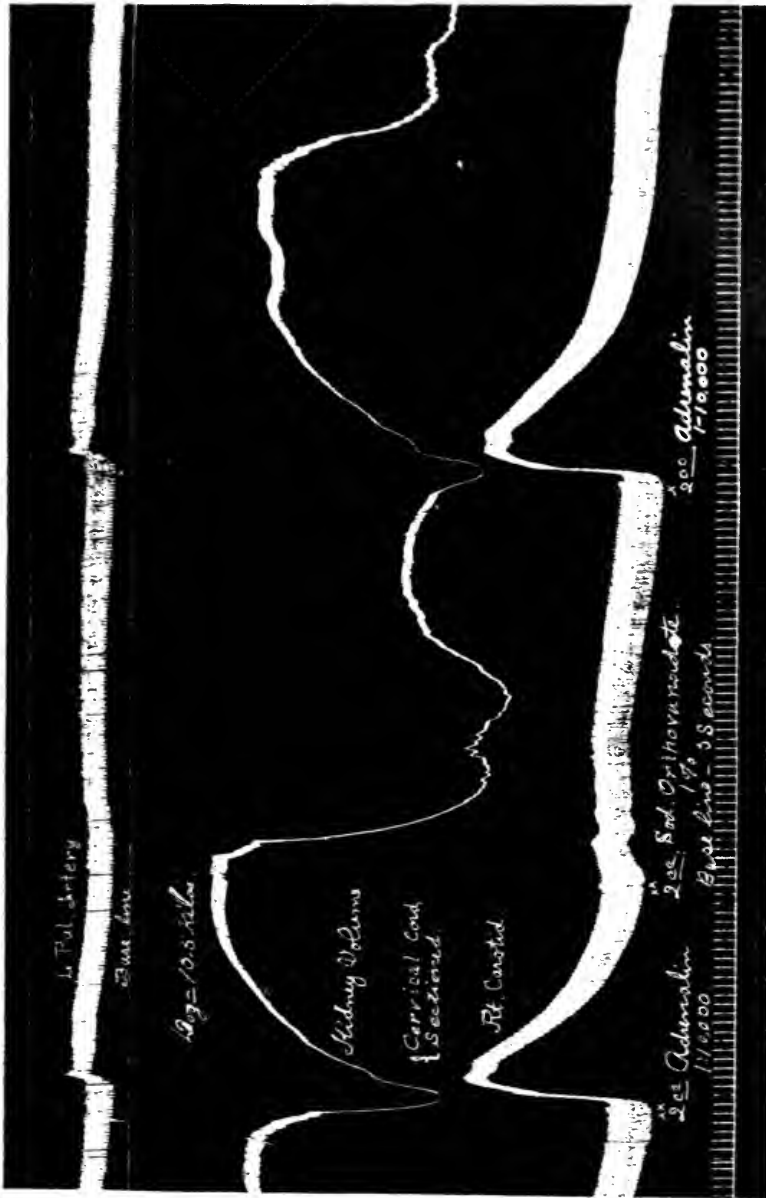


FIG. 1. LEFT PULMONARY BLOOD-PRESSURE, KIDNEY VOLUME, AND CAROTID TRACING FROM A DOG. CERVICAL CORD SECTIONED

Injection of adrenalin caused a rather marked abrupt rise in pulmonary pressure while vanadium produces a moderate, slow and prolonged increase of pulmonary tension. The metal greatly constricts the kidney volume and induces an irregular form of contraction of the vessels. Later a second injection of adrenalin gives a small preliminary renal contraction followed by a marked direct dilating action of the drug. This appears to be due to a direct stimulation of the vasodilator myoneural junctions. A little later the adrenalin action wears off and the vascular contraction due to the vanadium again appears. A strictly analogous action occurs on the bronchioles and on the intestinal peristalsis.

increased pulmonary pressure produced by injections of epinephrine, although in this case a direct stimulation of the heart muscle must also occur (fig. 1). This obstruction to the pulmonary venous outflow undoubtedly has much to do with the rise in pressure in the pulmonary artery. Third, a direct constricting action of the metal on the lung capillaries also helps to increase the pulmonary pressure. This can scarcely be shown directly by recording the pressure in the pulmonary artery by means of a mercury manometer as described above, for the other factors concerned would directly influence the results obtained. But I have demonstrated the direct action of the substance on the pulmonary capillaries by perfusion experiments in which a lobe of the lung was removed and perfused with Ringer's solution. The flow of the solution through the lung lobe was interrupted by means of a device operated by a water motor and the solution was warmed to body temperature by a water heater. It was found that the addition of a small amount of vanadium to the perfusion fluid would promptly decrease the rate of outflow from the vein to one-fourth or one-fifth of its former amount, and in some instances the constriction was even greater than this. There accordingly appears to be no room for doubt that the metal causes a direct constriction of the pulmonary vessels. It should be added that these phenomena occur in animals in which the cervical cord has been sectioned or in those from which the whole head has been removed. The action of the metal is therefore peripheral. It sometimes occurs that in animals in which the cervical cord has been cut the injection of vanadium does not produce any very considerable rise in systemic pressure (fig. 1). At first sight this would appear to indicate an action of the substance on the medullary vaso-constrictor center. This, however, is not the case for oncometric tracings of the kidney or spleen, taken at the time when the carotid pressure undergoes only a small rise, may show a most profound and extensive vaso-constriction. This is well shown in figure 1. The shrinkage in volume of the kidney, spleen, etc., under vanadium in these cases may be very greatly in excess of that produced by epinephrine but the latter substance causes a much greater systemic rise. The cause of this



difference I believe to be a variation in the extent of action on different vascular areas of the body. It seems that vanadium simply displaces a large amount of blood from the abdominal organs and drives it out into the peripheral vessels which are thereby dilated in these cases while epinephrine tends to cause a constriction in a much more extensive area of the vascular system. The difference in cardiac action under these two substances may also play a small part in the general results. And probably the difference in vascular action in the lungs may influence the general systemic pressure somewhat. Probably the general loss in vascular tone produced by separation of the vasomotor centers of the medulla from the cord leaves the general vasculature of the body in such a condition that drugs which act peripherally, and with a considerably greater force on certain areas than on others, may quite readily give somewhat variable results on systemic pressure from a sort of promiscuous shifting about from one region to another of rather large accumulations of blood.

#### THE BRONCHIAL ACTION

In examining this action I have used a number of different methods but need only consider one at this time. This is essentially the method used by Dixon and Brodie.<sup>2</sup> The animal (dog) was etherized and the chest was opened by a median incision, the two halves of the sternum being pulled widely apart and tied in order to fully expose the lungs. The upper lobe of the right lung was then lifted up carefully and with the least possible manipulation was placed inside a special plethysmograph. A perfectly regular artificial respiration was maintained after the chest was opened. The air and ether vapor were warmed by means of a special device containing a small incandescent bulb. The plethysmograph differed considerably from the form used by Dixon and Brodie. It was made of brass, in the form of a somewhat flattened cylinder. The outer end was closed by an air tight glass window through which the lung could be watched when placed inside. The inner end of the cylinder was constricted somewhat, resem-

<sup>2</sup> Dixon and Brodie: *Journal of Physiology*, 1903, xxix, no. 2, p. 97.

bling the surface which would be produced by flattening the frustum of a cone. The small end, which was placed around the base of the lung, carried a flange around its outer border. The greatest diameter of this small end was about 52 mm. and the shortest diameter about 42 mm. The large end of the instrument measured about 70 by 44 mm. The total length was about 95 mm. A sheet of rubber dam with an opening about 2 cm. in diameter in the center was tied over the small end of the plethysmograph. In placing the lung lobe within the plethysmograph the edges of the opening in the rubber dam were seized and stretched out laterly until they could be rolled back over the flange on the outer edge of the end of the plethysmograph. This left the whole end of the instrument open to receive the lung lobe which was then carefully lifted up and slipped within the instrument. The edges of the rubber dam were then gently rolled down again over the front of the flange and allowed to close in around the pedicle of the lung lobe. The whole process was quite easy and simple and required only a short time. Certain precautions, however, had to be taken. When the rubber dam was tied on to the plethysmograph care had to be used to see that the opening in the dam was approximately the same size in diameter as the pedicle of the lung lobe. If the opening in the dam were too small then the pressure on the pulmonary vessels would obstruct the blood flow, and if the opening were too large air would pass in and out around the pedicle of the lung. A large tambour with a deep bowl was used for recording the movements of the lung. A small tube soldered into the wall of the plethysmograph served to receive the rubber tube leading to the tambour.

It was found that the action of the metal varied a good deal on different animals. This in general was true of a considerable number of other drugs and the conclusion seemed fully justified that much of the variation was due to differences in the pulmonary susceptibility of different animals to the drugs. This also held good for electrical stimulation of the vagus nerves. I have also found this to be perfectly obvious by other methods than the one here described. It is quite easy to demonstrate a considerable variation in the extent of the inhibitory action of the vagus

nerve on the heart by electrical stimulation in even a small number of animals (of the same species). But it seems quite evident that very much greater variation in the extent of control by the vagus nerves on the bronchial musculature exists when the nerve trunks are electrically stimulated than is the case with the cardio-inhibition. Practically the most common difficulty which one meets in experiments of this kind is a failure of the lungs to show any response whatever, either of constriction or dilatation, to electrical stimulation of the vagi. In these cases one always feels sadly uncertain as to how much weight he should lay on any of the results of the drug reactions which he may later obtain from the animal. For one is constantly menaced by the fear that some slip or flaw in the technique which he may be entirely unable to foresee or detect, may be the cause of his failure to obtain the classical results. I perhaps, might suggest that in my experience it is absolutely imperative for one to perform a very considerable number of preliminary experiments of this kind before he can obtain results that he feels positively certain are reliable. Generally I have found the response of the lungs to the action of drugs to be much more certain and reliable than is the response to electrical stimulation of the vagi. By carefully checking the action of one drug with that of another it is usually possible to arrive at fairly reliable conclusions.

There are a large number of questions which might arise in regard to the accuracy and interpretations of the results obtained by this method. Most of these have been fully investigated and described by Dixon and Brodie<sup>3</sup> and need not be discussed here. Suffice it to say that the artificial respiration must be carefully regulated and the amount of anaesthetic used must be reduced to a minimum, for the volatile anaesthetics themselves may paralyze the endings of the vagus nerves in the lungs. In such cases as these negative drug reactions may signify nothing.

Briefly stated it was found that the intravenous injection of vanadium under the conditions above described caused a rather marked decrease in the amplitude of the respiratory excursions.

<sup>3</sup> Loc. cit.

This decrease was generally of a prolonged character and persisted for a period of five to twenty minutes, or even longer, if left to run its course. It was due to a moderate persistent constriction of the bronchial musculature and began a short time after the injection of the drug and reached its maximum at about the same time as the greatest vaso-constriction was reached in the abdominal organs. The total amount of blood in the lung lobe also increased slightly at the same time as shown by a slight rise in the lower limiting line.

The extent of the bronchial constriction was by no means so great as that produced by pilocarpine. Nor was the onset so abrupt as with the latter drug (fig. 2). In the case of both drugs, however, the injection of epinephrine would cause a prompt relaxation of the bronchial spasm. The action of the epinephrine in this case served quite well not only as a double check on the action of both of the other drugs but also as a positive proof that the technique and the method of procedure were wholly reliable. And further it can quite easily be shown that when a bronchial constriction has been induced by the injection of a vanadium solution, then the administration of atropine to the animal causes a prompt partial dilatation of the bronchioles (fig. 3). If a normal animal be treated with atropine until pilocarpine is no longer able to produce any bronchial constriction then the administration of vanadium will still induce a clearly demonstrable constriction of the bronchioles. In the latter instance, however, the later injection of adrenalin fails to cause any dilatation, and the constriction produced by vanadium in an atropinized animal is of only about half the extent of that which could have been produced in the animal by vanadium before the atropine was administered (fig. 3).

#### THE POINT OF ACTION OF VANADIUM

The results described in the preceding paragraph throw a good deal of light upon this question. It is quite probable that some other metallic substances may act upon the living mammalian tissues in a manner similar to that of vanadium. Consequently a

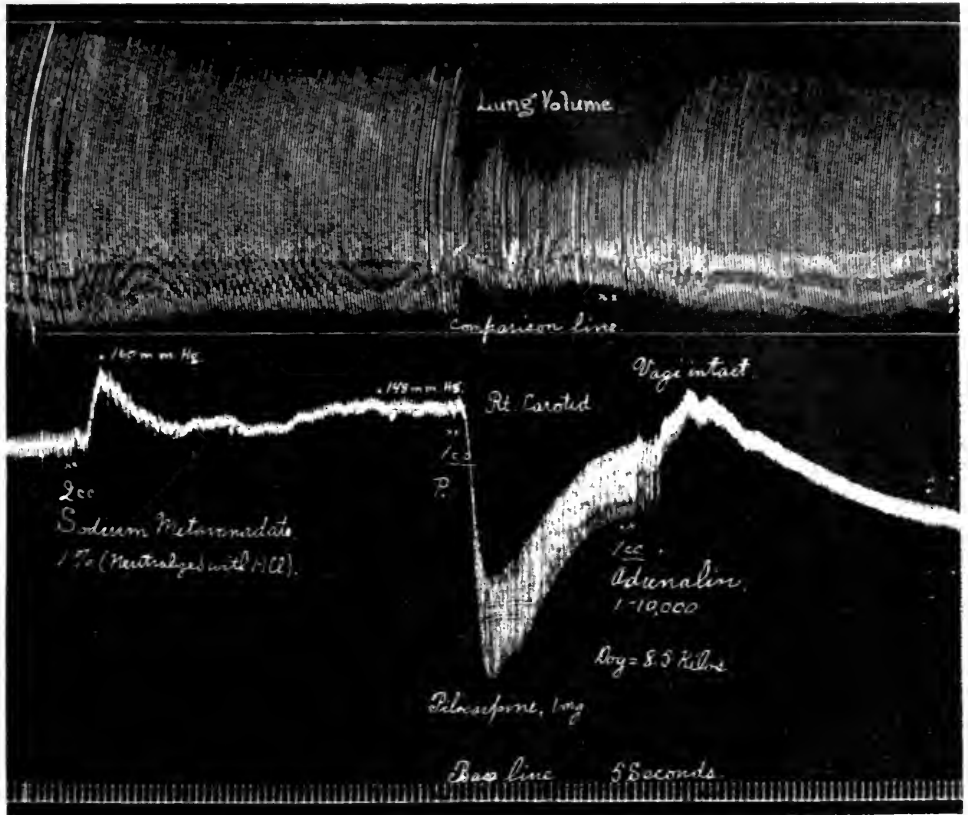


FIG. 2. LUNG VOLUME (UPPER LOBE OF RIGHT LUNG) AND RIGHT CAROTID PRESSURE TRACINGS FROM A DOG

Injection of sodium metavanadate caused a rise in blood-pressure and a decrease in the lung excursion. There is also a slight increase in the total volume of blood contained in the lung as shown by the rise in the lower limiting line of the plethysmographic record. Upstroke represents inflation of the lung lobe. Later 1 mg. of pilocarpine was injected and a much more extensive decrease in lung excursion follows. This decrease represents the combined action of the vanadium and the pilocarpine. A little later 1 cc. of 1-10,000 adrenalin was injected and the lung excursion promptly increased. A larger dose of adrenalin might have completely overcome the action of both the vanadium and the pilocarpine and restored the normal amplitude of the lung movements.

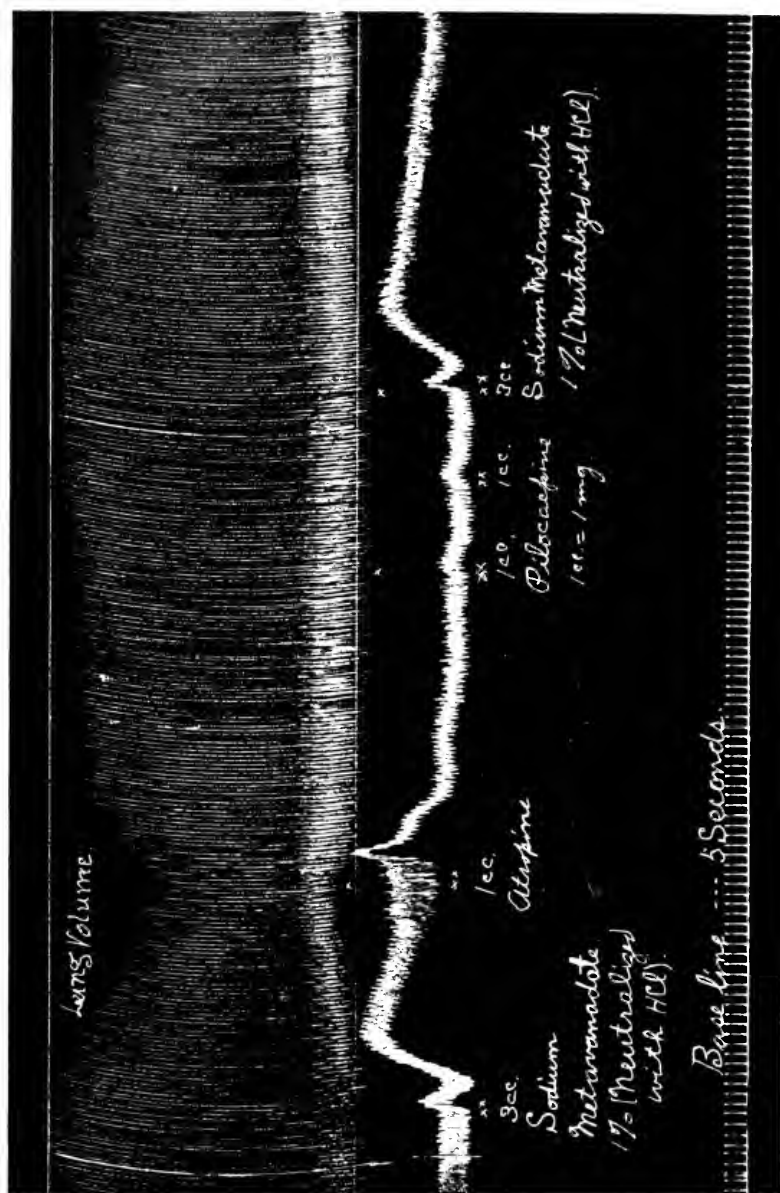


FIG. 3. LUNG VOLUME AND CAROTID BLOOD PRESSURE TRACINGS FROM A DOG

Injection of sodium metavanadate caused a decrease in the respiratory excursion. This was at once partially removed when atropine was injected. Later 2 mg. of pilocarpine were given without effect showing that the atropine had paralyzed the bronchoconstrictor nerve endings. Finally sodium metavanadate was again injected and the bronchioles were constricted about half as much as they were by the same amount of vanadium before the administration of atropine.

solution of the manner of action of this metal may have a sort of comparative value. When in a normal animal vanadium causes a bronchial constriction then this constriction may be due to a stimulation of several different structures, namely, the medullary centers, peripheral ganglia on the vagi nerves, the endings of the nerves in the bronchial muscles, upon the muscle fibers themselves or from reflex influences. Generally the metals which cause a contraction of smooth muscle at all are believed to act directly on the muscle fibers. It appears that the action of vanadium is almost wholly peripheral in character and that it stimulates both muscle and nerve endings. A number of different facts seem to bear this conclusion out quite clearly. But the methods required to demonstrate this point are not wholly free from objection. The fact that vanadium causes about twice as marked a bronchial contraction in a normal animal as it does in the same animal after the administration of atropine indicates at once both a muscular and a nervous action. The greatest objection to this experiment is probably the question as to whether or not the metal may not have acted wholly on the muscle fibers in the first instance and that the atropine may have simply weakened the muscle fibers themselves and consequently they may thereafter react less readily to vanadium. This view, however, is not the one usually held with reference to the action of small doses of atropine. These reactions may occur in animals in which the central effects have been eliminated.

Further evidence in this direction is obtained by the action of epinephrine which causes a prompt partial dilatation of the bronchioles when they are tonically constricted by vanadium (fig. 4). A further interesting feature in this reaction is the fact that after the effects of the adrenalin have disappeared, which usually occurs in about one to three minutes, the constricting action of the vanadium again returns (fig. 4), showing quite clearly the persistency of the vanadium action. The bronchial constriction following the injection of pilocarpine is much more completely and permanently removed by adrenalin than is the constriction produced by vanadium. This probably indicates the relative differences in the points of attack of the two substances upon the bronchial tissues.

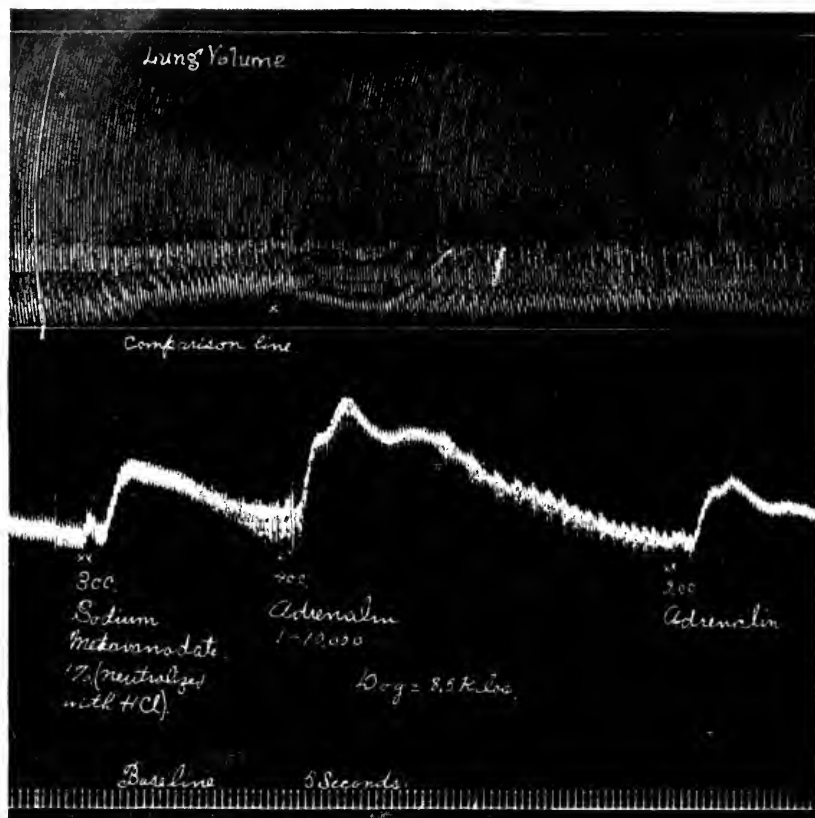


FIG. 4. LUNG VOLUME AND CAROTID PRESSURE TRACINGS FROM A DOG

Injection of vanadium causes pulmonary constriction which is mostly overcome by adrenalin, but as the adrenalin effects disappear the constricting action of the metal on the bronchioles again returns. A second dose of adrenalin again overcomes this. The readiness with which the stimulation of the myoneural junctions of the bronchodilator nerves by adrenalin can overcome the constricting influence of the vanadium is well shown.



The action of adrenalin in causing bronchial dilatation is believed to be due solely to stimulation of broncho-dilator nerve endings. Consequently it would be expected to be much more effective in counteracting the effects of drugs which act mainly, if not solely, on the broncho-constrictor nerve endings than those whose action is partly if not principally upon the muscle fibers themselves. But in this connection it probably ought also to be fully emphasized that often epinephrine caused a complete disappearance of the bronchial constriction produced by vanadium. After the effects of the epinephrine disappear in these cases, however, there is generally a slight return to the action of the previously given substance. In these cases it is probable that the endings of the broncho-constrictor nerves are specially susceptible to the action of vanadium.

An exact counterpart of this phenomena has already been mentioned in my former paper.<sup>4</sup> This consists in the marked opposing inhibitory action of ephinephrine against the effects of vanadium on the peristaltic movements of the intestines and the similar irregular and variable movements induced in the spleen and kidney by the metal. These phenomena and reactions are undoubtedly wholly comparable to those produced in the lungs. In each case epinephrine stimulates the myoneural junctions of the inhibitory nerves and thereby overcomes for a brief interval the stimulating action of the vanadium. The whole nature of this process indicates that in every instance the action of vanadium is the result of two factors, one nervous the other muscular.

A good example of the readiness with which a nervous inhibitory influence may overcome and be superimposed upon the regular action of vanadium is seen in the profound inhibitory action of epinephrine on the vessels of the kidney when they are contracted by vanadium (fig. 1). It would be difficult to find a more marked example of this phenomena than the one here presented. For by this means the existence and action of a vasodilator nervous mechanism may quite readily be shown, and the method might be used for even so simple a thing as a class demon-

<sup>4</sup> Loc. cit.

stration. When the kidney vessels are in a state of marked constriction, under the influence of vanadium then the injection of epinephrine may be followed by a preliminary transient increase in the vaso-constriction but this action rapidly passes off and then the kidney vessels promptly dilate. Without the epinephrine the vanadium constriction would be very much more prolonged. The dilation following the injection of the epinephrine is undoubtedly due to a direct stimulation of the vaso-dilator myoneural junctions. And this effect follows at some interval after the marked preliminary stimulation of the vaso-constrictor endings by the drug. After a short time, however, the action of the epinephrine ceases, apparently from a direct destruction of the epinephrine by the tissues. And then, exactly as occurs in the case of the bronchial musculature, the action of the vanadium again becomes visible. This secondary recurrence of the vascular action of the metal is often quite profound, and is plainly due to the persistent character of the vanadium reaction on the organs concerned. A second injection of epinephrine may again cause a dilatation of the vessels after the preliminary constriction. With care this can sometimes be repeated three or four times before the vanadium effects completely disappear from the vessels. And this is also true of the bronchioles. The promptness and effectiveness of this nervous vaso-dilatation during a vanadium constriction gives one the impression at once that the metal may act partly by stimulating the vaso-constrictor endings. In this probably no structures are weakened or paralyzed as occurs in the lungs when atropine is administered.

Further evidence upon this question has been sought in another line of experimentation. It seemed that the well-known action of ergotoxine might be utilized in this connection. A large dose of ergotoxine injected intravenously will promptly paralyze the vaso-constrictor myoneural junctions. This can easily be checked up by the injection of epinephrine. When an animal is treated in this manner and a small amount (20 mg.) of a neutralized sodium orthovanadate solution is injected into the femoral vein a rise in arterial pressure is still produced. The character of this rise is not, however, the same as that produced by vanadium in a normal

animal. It is considerably slower in reaching its maximum elevation but the duration of the rise is about the same as that in the normal dog. Generally the maximum elevation above the previous pressure reached after ergotoxine is less than occurs with the metal in the normal animal. It is difficult to determine, positively, the cause of the altered reactions. But the mere fact that a rise in pressure is produced after ergotoxine shows that the rise cannot be dependent solely on nervous influences. It must therefore be partly and perhaps mainly, of muscular origin. The disappearance of the rather abrupt character of the rise at the beginning may be due to the generally lowered tone of the blood vessels and a consequent sluggishness of general reaction to the drug. There is also a possibility that the toxicity of the ergotoxine itself may have directly weakened the muscle fibers of the vessel walls so that they no longer respond quickly to the vanadium. Notwithstanding these objections, however, I am inclined to believe that the action of the metal on the vessels is due both to a stimulation of the vaso-constrictor myoneural junctions and of the muscle fibers themselves. The latter certainly occurs, but unfortunately an indisputably positive proof for the former is not readily obtainable. On the contrary it is equally difficult to prove that a nervous stimulation does not occur. In this connection it should be noted that the addition of vanadium to Ringer's solution which is perfused through the pulmonary vessels causes a constriction. And in a former series of experiments in which vanadium solutions were injected by way of the femoral veins into dogs I failed to observe any movements either of the pupil or of the uterus. Possibly the physiological state of the latter organ in the animals used, or the great dilution of the vanadium in the blood circulating through the organs concerned may have influenced the results in both these later instances.

In order to further elucidate this point I have performed a series of perfusion experiments. A kidney (or spleen) was removed from a dog and after the insertion of the required cannulas into the kidney vessels and ureter the organ was placed in a small air-tight box which was connected by rubber tubing to a record-

ing tambour which marked on a revolving drum. Ringer's solution was used to perfuse the organ. The solution was warmed in a small water heater and the flow into the organ was regularly interrupted at about the same rate as that of the heart beat by means of a water-motor. A current of air was constantly forced through the Ringer's solution. When all arrangements were completed and a good artificial "pulse" tracing was being recorded by the tambour, an injection of ergotoxine phosphate (Burroughs, Welcome and Company) solution was added to the perfusion fluid. An intense contraction of the kidney volume was at once recorded and the pulse-like vibration of the writing point decreased to a minimum. A little later the kidney was flushed out with fresh Ringer's solution and an injection of epinephrine (adrenaline, Parke, Davis and Company) was added to the perfusion fluid. No contraction of the kidney volume could be obtained with even very large quantities of epinephrine, thus indicating that the vaso-constrictor myoneural junctions had been paralyzed by the ergotoxine. The organ was again flushed out with fresh Ringer's solution and an injection of vanadium was given. A medium shrinkage in kidney volume was promptly recorded. This appears to show that vanadium undoubtedly acts directly on the muscle fibers of the vessel walls. But since the contraction produced by vanadium after ergotoxine was in every instance much less than could have been produced by the vanadium in the normal organ we are again confronted with the possibility that the metal in the unpoisoned organ may directly stimulate the nervous vaso-constrictor mechanism. And considering all the evidence at hand I am greatly inclined to believe that this is what the metal actually does. The most serious objection to the above experiment is, of course, the possibility that the ergotoxine or the epinephrine or both may have so weakened the vessel walls that when the vanadium is injected only a moderate response can be obtained. This is the same objection as was mentioned in the case of the use of atropine to paralyze the broncho-constrictor nerve endings after which treatment vanadium causes a considerably less marked broncho-constriction than was produced in the normal lung. By way of summary it may be stated that the decrease in broncho-constriction produced by vanadium after atropine,

the decrease in vaso-constriction produced by vanadium in the excised perfused kidney after ergotoxine, the very striking vasodilatation which can be readily superimposed (after a short preliminary increase in vaso-constriction) on the renal vaso-constriction produced by vanadium in the intact animal and the promptness and decisiveness with which the vanadium vaso-constriction again reappears in the kidney after the transient dilating effects of the epinephrine have disappeared all may be considered as pointing strongly toward a nervous as well as a muscular action by vanadium. It would appear that with very large or often repeated doses of vanadium the muscular action of the substance comes, after a time, to overshadow and displace the nervous stimulation. The cause of this may be a partial paralysis of the nervous structures or it may be due to a gradual persistent increase in the muscular stimulation produced by the metal. Aside from the consideration of this later action of vanadium, however, it seems that the evidence in favor of a direct stimulation of myoneural junctions in certain forms of smooth muscle by vanadium is more extensive and convincing than any which has been heretofore adduced respecting a similar action by other metals. As was mentioned earlier in this article the above conclusion may be of interest in connection with the study of the pharmacological action of other metals. The peculiar and extreme irregular alternate constriction and dilatation which is induced by vanadium in the vessels of such organs as the kidney or spleen seem to show at once some form of nervous activity. For such irregular and profound variations from the normal conditions of the vessels do not seem to be even imitated by other metallic substances (and possibly not by the vegetable poisons).

A matter which may be of some general interest but which is not indicated in the general title of this article may be briefly mentioned here. From time to time during the last fifteen or twenty years many various claims have been made regarding the antiseptic or bactericidal action of vanadium. Many different forms of vanadium-containing compounds have been used in the belief that they would prove of marked benefit in various infectious conditions either when applied locally or else when taken internally. A consideration of these facts have led me to believe

that a few simple experiments in this direction might be of some value. Lyonnet, Martz and Martin<sup>5</sup> in 1899 performed a series of experiments on this subject. They found that sodium metavanadate in the proportions of one to one thousand exercised almost no antiseptic power on typhoid bacilli. In the present experiments I have used five forms of bacteria, namely, *B. coli*, *B. proteus vulgaris*, *M. albus*, *B. pyocyaneus*, and *B. prodigiosus*. I have found that none of these forms are appreciably hindered in their growth by the presence of sodium metavanadate in the proportions of one to one thousand. Careful control series in plain media and in media to which bichloride of mercury was added in the proportions of one part to one thousand of the media were also carried out. In no instance was a growth ever obtained in the presence of the mercury. This shows that sodium metavanadate (which is more toxic than the orthovanadate) is by no means so effective an antiseptic as is bichloride of mercury. A concentration of sodium metavanadate so strong as one to one thousand parts of blood would be rapidly fatal to mammalian forms. These experiments coincide exactly with the earlier findings of Lyonnet, Martz and Martin.

Perhaps at this point another phase of the subject should also be mentioned. The quantity of vanadium required to produce marked bronchial constriction or a rise in pulmonary pressure is greatly in excess of any quantities which could be tolerated medicinally. The susceptibility of these structures to the action of vanadium is, indeed, very much less than that of the vessels of the abdominal organs. In dogs weighing 8 to 12 kg. it is usually necessary to inject from 1 to 2 cc. of a 1 per cent solution (10 to 20 mg.) of sodium orthovanadate intravenously to obtain a perfectly satisfactory result. This quantity of vanadium is much larger than could be given to a patient. Consequently this action on the bronchial musculature or pulmonary circulation could scarcely be used in any sense whatever as a basis for the scientific treatment of pulmonary tuberculosis with salts of vanadium.

<sup>5</sup> Lyonnet, Martz and Martin: *De l'emploi des sels du vanadium*, Lyon Méd., 1899, xc, pp. 289-302. Also *Journal of the American Medical Association*, July 24, 1909, p. 309.

## A STUDY OF THE ACTION OF THE HEART IN ANAPHYLACTIC SHOCK IN THE DOG

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In a former communication<sup>1</sup> we published the results of a series of experiments on the physiology of anaphylactic shock in the dog and drew the conclusion that the fall in blood pressure characteristic of the anaphylactic reaction in that animal was due to paralysis of the peripheral vaso-motor system. Attention was called also to the fact there was no evidence of any primary alteration in the heart action and that the slight increase in pulse rate and decrease in pulse volume occurred only when the blood pressure level had fallen materially. Studies of the distribution of the blood showed an accumulation of the blood in the larger venous trunks, and particularly in the splanchnic area. Such being the case a markedly diminished volume of blood was being returned to the right side of the heart and a correspondingly diminished output from the left ventricle resulted. Our explanation we considered to be amply sufficient to account for the phenomena characteristic of anaphylactic shock in the dog.

To our knowledge no reports of experiments have been published which demonstrate either that a condition of vaso-constriction exists or that alteration of cardiac activity plays a primary or even a secondary part in the abrupt lowering of blood pressure that is the characteristic and practically the only phenomenon exhibited when an intoxicating dose of horse serum is injected into a sensitized dog under ether anesthesia. Although no evi-

<sup>1</sup> Pearce, R. M., and Eisenbrey, A. B., The Physiology of Anaphylactic Shock in the Dog. Jour. Infect. Dis., 1910, vii, 565.

dence has been adduced to furnish reasons for altering our views in regard to the physiology of the fall in blood pressure, the differing conclusions of writers who have worked with other animals impel us to present the following work in corroboration of our former conclusion as to the negative rôle played by the heart in the reaction in the dog.

Since the publication of our earlier results numerous articles have appeared which demonstrate conclusively that the anaphylactic reaction exhibits the widest variations in its manifestations according to the species of animal used. The predominating and almost specific action on the smooth muscle of the bronchioles of the guinea pig, the very definite action on the heart muscles of the rabbit and upon the vessels of the pulmonary circulation of the cat indicate that considerable caution must be observed in attempting to apply the findings in one species of animal to an explanation of the phenomena encountered when working with an animal of another species.

While the balance of evidence supports the idea that smooth muscle (or at least involuntary muscle) is the tissue primarily affected in the anaphylactic reaction there are as yet no grounds for the belief that it must necessarily be affected in the same manner or to the same degree or that the regional involvement must be similar in all animals. On the other hand, the work previously mentioned indicates that the contrary is the case. Even though the usual effect has been found to be the production of a state of contraction in smooth muscle it has by no means been shown that this is invariably the case.

In this connection much stress has been laid upon the slight and momentary rise occasionally found to occur immediately before the abrupt fall in blood pressure in anaphylactic shock in dogs, which has been interpreted as indicative of vaso-constriction. This effect is not constant nor must it be explained solely on the basis of a vaso-constriction. In many normal animals with a good general blood pressure the fairly rapid intravenous injection of even a relatively small bulk of fluid (5 cc. of normal salt solution, dog's urine, peptone solution or horse serum) is often sufficient to cause a similar brief rise, yet such rises are not



generally attributed to a specific vaso-constrictor action. Moreover, the ensuing fall in the blood pressure is abrupt and certainly does not resemble the fall produced by the type of deficient cardiac action that might result either from anemia or asphyxia of the heart muscle, nor is there evidence of a disturbance of heart action suggestive of a toxic action on the muscle or on its innervation. Moreover, the fall in blood pressure is not produced by the injection of horse serum unless the animal has been sensitized by a previous injection of serum.

The work of Auer<sup>2</sup> on the rabbit showed that marked differences exist between the type of anaphylactic reaction in that animal and the types of reaction characteristic for the guinea pig<sup>3</sup> and for the dog.<sup>4</sup> In two articles by W. H. Schultz, appearing in this journal, statements have appeared which deserve a word of criticism in view of the facts generally accepted as established by experimentation in the physiology of anaphylaxis. In the first place an obvious attempt is made to apply the conclusions based on experiments on cats to the reaction in other species of sensitized animals, although many of the experiments were made on unsensitized cats. Furthermore it is stated, without the publication of any experiments in support of the contention, that the conclusions of many workers in regard to the physiology of the anaphylactic reaction in the dog are incorrect. In the first article,<sup>5</sup> in a footnote on page 384, speaking of unpublished results of experiments on cats, dogs and guinea pigs, the statement is made that "It however was proved that (4) The fall in blood pressure observed in all anaphylactic animals is attributable primarily to the heart, the lethal dose of the protein reacting with the receptive substance of (1) the smooth muscles and (2) possible also with that of the cardiac muscle itself."—W. H. S. In the second

<sup>2</sup> Auer, J., Lethal Cardiac Anaphylaxis in the Rabbit. *Jour. Exper. Med.*, 1911, xiv, 476.

<sup>3</sup> Auer and Lewis, *Jour. Am. Med. Assoc.*, 1909, liii, 458; also *Jour. Exp. Med.*, 1910, xii, 151.

<sup>4</sup> Biedl and Kraus, *Wiener. klin. Wehnschr.*, 1909, xxii, 363.

<sup>5</sup> Schultz, W. H., and Jordan, H. P. E., *Physiological Studies in Anaphylaxis. III. A Microscopic Study of the Anaphylactic Lung of the Guinea Pig and Mouse. Jour. Phar. and Exper. Therap.*, 1911, ii, 375.

article<sup>6</sup> on pages 311 to 312, in a description of the anaphylactic reaction in the cat, appears the statement:

In spite of the contentions of Arthus, Friedberger, Biedl and Kraus, Pearce and Eisenbrey, Auer, and nearly every writer on this subject that horse serum causes vaso-dilatation, it can be shown to cause vaso-constriction. If this be so there is no reason for hesitating to say that the heart and lungs are for the most part responsible for the low blood pressure, and that the venous congestion observed is for the most part a passive effect brought on by a block in the circulation, making it impossible for the right heart to empty itself.

It is not our intention to deny that vaso-constriction of some degree (although to our knowledge it has not been demonstrated) may occur somewhere in the circulatory system of the dog at some time during the anaphylactic reaction, but we do contend that it is not a vaso-constriction of the pulmonary circulation leading to a damming back of the blood into the right heart that is the primary or essential factor in the production of the fall of blood pressure characteristic of the reaction in that animal, and that furthermore we believe we have shown that this fall in blood pressure is not attributable primarily to the heart.

Even in the absence of evidence contrary to that on which our conclusions were based, it has seemed advisable in view of the above statements to publish in corroboration of our earlier explanation the following experiments, in which direct tracings of cardiac action were taken.

Dogs averaging from 7 to 15 kg. in weight were given subcutaneous injections of 5 cc. horse serum and after eighteen to twenty-five days were anesthetized and the physiological experiments performed. In all cases the animals were under full ether anesthesia administered by intratracheal insufflation and by this method the artificial respiration necessary after opening the thorax was maintained.

The blood pressure was recorded from the right femoral artery by mercury and Hürthle membrane manometers and, after opening the thorax in the mid line, the heart action was recorded by

<sup>6</sup> Schultz, W. H., *Physiological Studies in Anaphylaxis. IV. Reaction of the Cat Toward Horse Serum.* Jour. Pharm. and Exper. Therap., 1912, iii, 299.

the Cushny myocardiograph. The intoxicating injection of 5 to 6 cc. of horse serum into the left femoral vein was made with due caution as to the temperature of the serum and the rate of introduction. The recording apparatus was arranged as far as possible in alignment and corresponding points on the tracings were carefully measured from the records made by the recording arms of the apparatus when the drum was at rest.

In four experiments of this nature the average time of the appearance of the reaction after the injection was twenty-eight seconds and the average of the maximum fall in blood pressure was 50 mm. of mercury. This fall is somewhat less than that usually seen when intact animals are used but as the blood pressure after the thorax is opened averages only about 90 mm. the fall is relatively as great as in the intact animal.

In two experiments a slight transient rise amounting to not more than 8 mm. preceded the beginning of the fall and it may be noted in this connection that similar rises occurred also in several experiments in which for some unknown reason the anaphylactic reaction did not result.

The appearance of the myocardial tracings was somewhat marred by the fact that no attempt was made to do away with respiratory movements. In none of these experiments did any change in the character of the myocardiogram occur until after the blood pressure had fallen to a marked degree and even then the tracings do not show a noteworthy change in rate or in lessening of the amplitude of the cardiac contractions. In fact, in most instances, there was a definite tendency to a slight increase in the range of contraction.

The change in position of the recording arm of the myocardiograph to a lower level occurs after the blood pressure has begun to fall and is due to a factor significant in itself and easy of direct observation. Following the accumulation of blood in the splanchnic area and the fall in blood pressure, the volume of the right ventricle may be seen to decrease and the ventricular wall collapses inward so that the arms of the apparatus which are attached to the heart muscle are passively approximated even during diastole and the recording lever is therefore slightly depressed. There

is, however, no diminution of the activity of the ventricular contractions and the condition must be explained on mechanical grounds by the fact that the blood returning to the right side of the heart is markedly reduced in amount. The right auricle also presents the appearance of being incompletely filled. Our contention that the bulk of the blood is comparatively stationary in the large venous trunks thus receives further support, for in view of this condition in the right side of the heart it is not reasonable to assume that the deficient output of the left ventricle and consequently lowered blood pressure is produced by a damming back of the blood in the right heart by reason of vaso-constriction in the pulmonary circulation or to the inefficient action of a heart rendered anemic by constriction of its coronary vessels. Nor is there any indication that the heart muscle is directly affected by the "toxic" dose of the serum (see figs. 1 and 2).

In the course of a series of experiments<sup>7</sup> on conditions of low blood pressure in dogs, anaphylactic shock was one of the conditions studied and it was repeatedly found that when a sufficient volume of fluid was delivered to the right side of the heart, that organ was fully capable of normal activity as long as such a delivery was maintained. If, however, such measures were used after the organ had been permitted to undergo for too long a time the anemia consequent upon the low blood pressure level, it did show evidence of deficient action.

It is of interest to note the striking resemblance between anaphylactic shock in the dog and the conditions produced by the injection of dog's urine or peptone solution, both in the character of the blood pressure fall and in the evidently negative rôle played by the heart in that fall. The only difference to be noted is merely one of degree and a tendency to spontaneous recovery (see figs. 3 and 4) after the action of urine or peptone.

<sup>7</sup> Pearce, R. M., and Eisenbrey, A. B., A Study of Experimental Conditions of Low Blood Pressure of Non-Traumatic Origin. *Archiv. of Int. Med.*, 1910, vi, 218.

## CONCLUSIONS

Myocardiograph records show that the functional activity of the heart is not primarily affected by the injection of the "toxic" dose of horse serum in sensitized dogs. The rate and range of contractions remain unchanged even after the general blood pressure has started on its abrupt fall. Subsequent changes in the character of the myocardiograph tracings are due to the incomplete filling of the right side of the heart consequent upon the accumulation of the blood in the larger venous trunks and particularly those of the splanchnic area.

Inspection of the heart shows a marked decrease in the size of the right auricle and ventricle. The right ventricular wall appears flabby and collapsed during diastole but contracts during systole in rate extent and regularity as it does before the injection.

Early in the period of low blood pressure the heart has been found to be functionally unimpaired when measures are taken to supply a sufficient volume of fluid to the right side of that organ. Later the heart muscle may suffer from anemia.

No evidence is at hand to indicate that any possible vaso-constriction of the coronary or pulmonary vessels or action on the heart muscle itself is responsible for the characteristic fall in blood pressure in anaphylactic shock in the dog. These conclusions are based on experiments on anaphylactic shock in the dog and there is no intention to apply them to the phenomena of the anaphylactic reaction in the cat, rabbit, guinea pig or any other animal.

Incidentally it has been shown that the fall in blood pressure following the injection of dog's urine or peptone solution into the normal dog is likewise independent of action on the heart.

## EXPLANATIONS OF FIGURES 1, 2, 3, 4

From above downward the tracings are: myocardiograph, blood pressure, base line, membrane manometer, base line, signal, time in seconds. The down strokes of the myocardiograph tracing represent cardiac contractions. The perpendicular lines were drawn arbitrarily through the blood pressure curves at points where the fall began. Corresponding points in the myocardiograph and Hürthle manometer tracings were measured and are indicated by crosses where the recording levers were not in accurate alignment.

Figs. 1 and 2 illustrate anaphylactic shock; fig. 3, the effect of dog's urine and fig. 4, the effect of peptone solution.

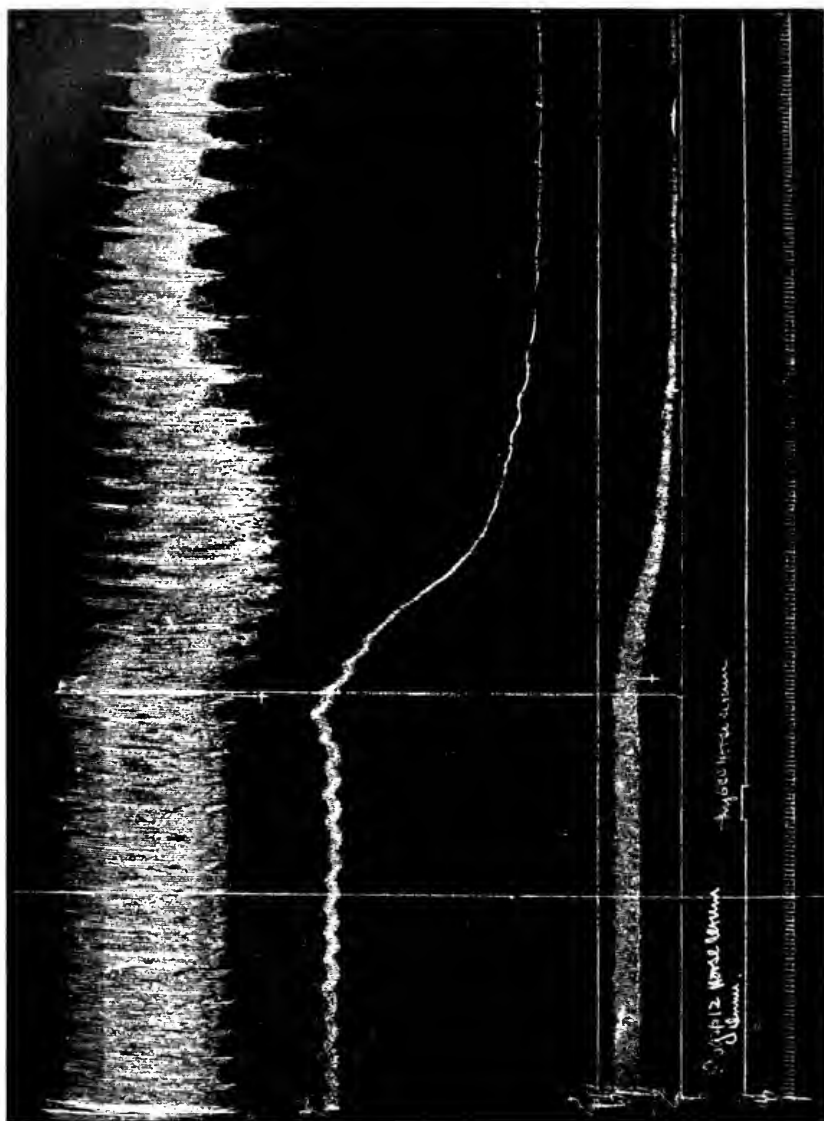


FIG. 1. ANAPHYLACTIC "SHOCK"

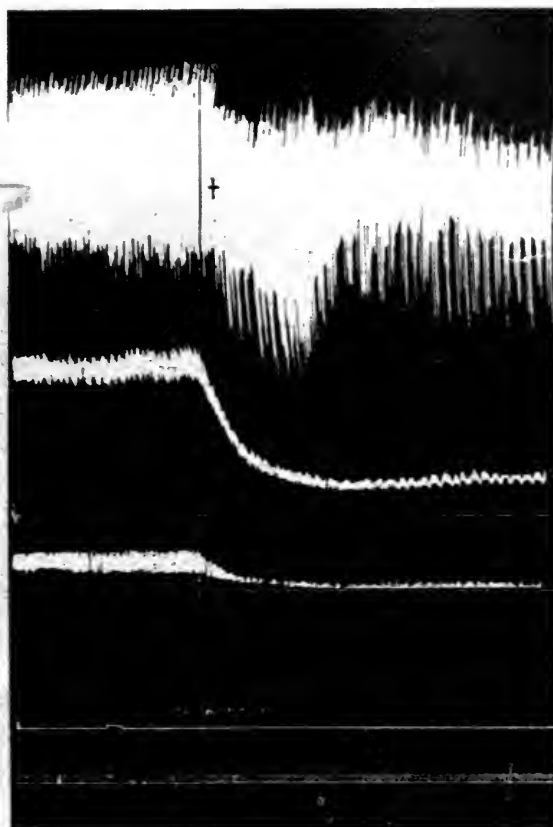


FIG. 2. ANAPHYLACTIC "SHOCK"

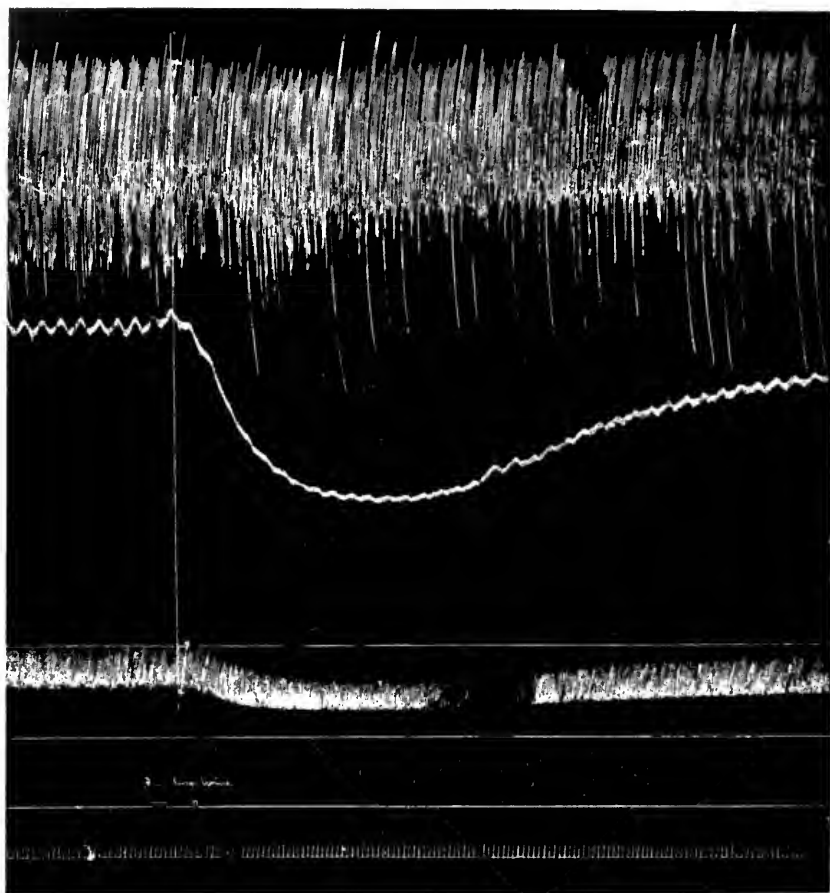


FIG. 3. DOG'S URINE



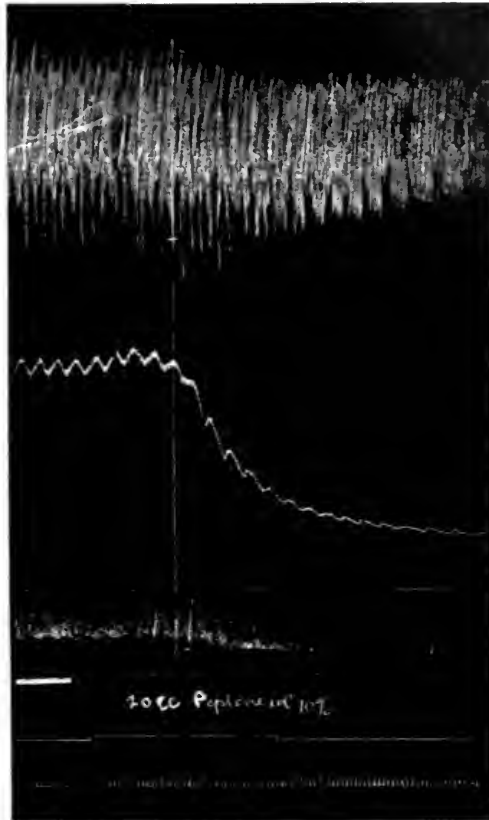


FIG. 4. PEPTONE SOLUTION



## ON THE PRODUCTION OF EXPERIMENTAL CEPHALIC COMA

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During the course of some recent experiments I have found it necessary to use a considerable number of decerebrate animals. In the year 1909 Sherrington<sup>1</sup> published a method for the production of a "spinal" animal, but in my own work I have met with certain objections to Sherrington's process which have led me to devise a new method for the production of these specimens. The immediate availability and wide application to which the method may be put, both for research purposes and for ordinary student work or demonstrations, urges me to publish this brief description. I have so far used the method only on dogs, but I believe it may also be available for cats. It probably cannot be used advantageously on animals smaller than cats.

In a general way we may consider that the blood supply for the brain, medulla and upper part of the spinal cord is derived from four great arteries, the right and left common carotids, and the right and left vertebrals, and that the venous return from the organs within the cranial cavity is mainly by way of the jugular veins on either side. It seems quite evident that in dogs a considerable variation exists in different animals regarding the relative amount of blood carried by each of these arteries to the various parts of the cephalic portions of the central nervous system. Roughly speaking the terminal portions of the carotid arteries supply blood to the anterior and middle parts of the cerebrum

<sup>1</sup> Sherrington, C. S.: Jour. of Physiol., 1909, xxxviii, no. 5, p. 375.

(through the anterior and middle cerebral arteries) and the vertebrals furnish blood for the posterior part of the cerebrum (through the posterior cerebral artery), the cerebellum (through the various cerebellar arteries), the pons (through the basilar branches) and the medulla. Ultimately from the vertebrals are derived also the small anterior and posterior spinal arteries which are continued down the cord by means of communicating branches from the intercostal and lumbar arteries. Aside from this general vascular supply may be mentioned the venous plexus found posteriorly and laterally to the cervical portion of the spinal column.

If a few drops of chloroform be injected into one of the (intact) femoral arteries the substance will be at once carried out into the corresponding hind limb and thoroughly distributed through the capillaries. The result is immediate and profound. Within one or two seconds the muscles and tissues which have been reached by the chloroform become hard, firm and lifeless. The extreme rigidity and firmness of these muscles is quite similar to that of frozen tissues. The action appears to consist in a direct chemical coagulation of the living protoplasm of the cells. The death of the cells thus acted upon seems to be practically instantaneous. The condition of the coagulated tissues is apparently entirely permanent. In no instance have I ever seen the faintest indication of a return to the normal condition of the tissue throughout the course of the longest experiments. It was the utilization of this simple pharmacological principle which has made possible the method I have used.

The method may be briefly described as follows. The animal (dog) is rapidly anaesthetized with the least possible amount of ether. It is then quickly placed on the operating table and a tracheal cannula is inserted into the windpipe. Both carotid arteries and both vagi nerves are then isolated and ligatures are placed loosely around each structure. Usually the right carotid is connected with the kymograph. Next the median incision in front of the trachea is extended down to the manubrium of the breast bone. Haemostats are attached to the separated sternothyroid muscles about 1 inch from their sternal attachment. These muscles are then pulled apart and the trachea is exposed

down to the root of the neck. The internal and external jugular veins are then carefully isolated on each side and one ligature is placed around each pair and tied loosely, care being taken that the flow of blood through the veins is not interrupted at this time. For convenience I usually attach *haemostats* to the loose ends of these ligatures in order that I may be able to pick them up rapidly and draw the knots tight at a later stage of the process. Sometimes I simply ligate the innominate veins on each side and thereby avoid dissecting out the two veins (internal and external jugular) on each side. It is better to begin, however, by picking up both veins, since a dissection for the innominates carries one well down into the apex of the thoracic cavity. When these veins have been loosely ligated a dissection is made on the right side of the trachea down into the root of the neck. The anterior surface of the right longus colli muscle is exposed and by following its outer edge down to the lower border of the right transverse process of the sixth cervical vertebra the right vertebral artery will be found. At this point the course of the artery is cephalad, slightly backwards and slightly inwards. The vessel which is usually about one-third smaller than the corresponding carotid artery is surrounded by a small accumulation of fat and connective tissue at the apex of the thoracic cavity, where it passes above the lung. The artery is exposed and a ligature is placed around it and tied loosely. Usually I place a *serresfin* on the ends of this ligature. A good deal of variation is found in different dogs regarding the ease with which the vertebral artery may be found. Usually it is necessary to penetrate the fascia filling the cavity at the apex of the thorax. This makes no difference in the course of the experiment, however, for a pledget of cotton is at once rolled up and pushed down along the side of the trachea and beyond the vertebral artery. The cotton thus passes between the trachea and the artery and when packed below the vessel serves to hold it up out of the surrounding fascia and in an easily accessible position.

Occasionally when I wish to find the vertebral artery quickly and the dissection as above described does not readily bring it into view I simply dissect down directly into the tip of the apex of the

thorax and hunt for the subclavian artery. This is usually quite readily found and it is then an easy matter to define the vertebral branch and trace it directly forwards along the edge of the longus colli muscle to the point where it passes backward and enters the vertebral foramen of the sixth cervical vertebra. The available length of the artery is usually about 1 to  $1\frac{1}{2}$  inches. The dissection for this artery is the most difficult part of the whole process. With a little practice one can generally isolate the vessel in a minute or so after the thyroid muscles have been separated. In a good dissection the hemorrhage should be very slight.

When these vessels have been properly exposed and ligated the next step consists in the injection of the chloroform. It is imperative that certain precautions be taken in this process. Usually I employ a small dental hypodermic syringe holding about 2 cc. and having a fine point which is only about  $\frac{1}{4}$  inch in length. When everything is ready for the injection, care being taken to see that the animal is sufficiently well under the influence of ether not to struggle for a minute or two, the kymograph is started, an aneurism needle is passed under the exposed vertebral artery and a *bull-dog clamp* is placed on the left carotid artery. By means of the aneurism needle the vertebral artery is then lifted up a little and the point of the syringe passed slantingly through the wall of the vessel and into the lumen. It is very important to see that no chloroform is hanging on the point of the syringe in the form of a small drop at the time that the point passes into the vessel. If such should be the case this drop of chloroform will at once act upon the arterial wall and so weaken it that a hemorrhage will probably result. It is very necessary to be sure that the point of the syringe needle passes clearly into the lumen of the vessel. The syringe is then rather rapidly emptied and the chloroform is carried forward into the medulla, cerebellum and cerebrum. Generally 1 or 2 cc. of chloroform is sufficient to completely destroy the vitality of the brain and the upper extremity of the spinal cord. The immediate result of a successful injection is the prompt cessation of respiration and all voluntary movements and a marked fall in blood pressure. At the first indication that these results have been produced the ligatures around the jugular veins are lifted up and slight traction is made on each. This slight

pressure seems to stop the flow of blood from the head back toward the heart. If the injection has been fully successful the veins may at once be tied tightly. Usually I put a bull-dog clamp on the vertebral artery at the point where the syringe passed into the vessel. All anaesthetic can be at once omitted and artificial respiration is begun. The blood-pressure usually falls to about one-third or one-fourth its former height but after a little while may rise considerably. If the first injection is not wholly successful later injections may be made either into the left (intact) carotid or into the cephalic end of the right (ligated) carotid, or again into the right vertebral. This is seldom necessary, however, and in a successful experiment but one injection into the vertebral is required. It is sometimes surprising how small a quantity of chloroform may suffice to completely allay all signs of brain activity. In a few instances I believe I have accomplished this with not more than seven or eight drops of chloroform. Occasionally one does not succeed well with the first injection and a second or even several later ones are required. This, however, is probably due in nearly all cases to improper technique. And the commonest source of failure is the incorrect insertion of the syringe point into the artery. If the syringe needle is merely imbedded in the wall of the vessel then upon injection these tissues will swell and break down immediately. The best method is to lift up the vessel on the hook of the aneurism needle for a few seconds. This partially or completely closes off the lumen of the vessel and the cephalic portion of the artery tends to empty itself into the veins. The syringe point is then carefully and accurately inserted and the tension on the aneurism needle is relaxed a little. No hemorrhage around the syringe point should occur. As the blood is again seen to flow freely through the vessel and into the partially emptied cephalic portion the syringe is emptied, the chloroform being thereby rapidly carried forward in the blood stream.

It was mentioned above that a clamp should be placed on the left carotid artery just before the injection was made. A few words of explanation may be given in this connection. The right carotid had been previously interrupted when the arterial cannula was inserted. This left three arteries to carry blood to the brain. All three of these empty into the circle of Willis so that

the relative distribution of blood to the various parts of the cerebrum was probably not materially influenced. With the clamping of the second carotid artery, however, all the blood to the brain would be supplied by the vertebrals which unite to form the basilar artery before the circle of Willis is reached. If chloroform is injected into one vertebral it will therefore stand a very good chance to be distributed through the circle of Willis almost equally to all parts of the brain. This would probably not occur if one or both carotids were carrying forth their normal output of blood.

By a sort of reversal of this latter principle I have sometimes seen what appeared to be a destruction of the cerebrum while the medulla and perhaps portions of the cerebellum and pons remained intact. By careful injection into the carotid arteries alone, and with proper manipulation I suspect it may be readily possible to destroy the cerebrum and still leave the hinder portions of the brain intact. This method may be available for study of the functions of these or other parts of the central nervous system. The function and reflexes of the cord are fully preserved. I have not, however, made any very careful study of this phase of the subject. Sometimes I have seen what appeared to be somewhat abnormal results follow injection into the artery, and have been led to suspect that occasionally these results may arise from an anomalous formation of the circle of Willis, or possibly some other abnormal obstruction or distribution of the artery may have been present.

Certain well defined phenomena usually attend a perfectly successful injection. If the blood pressure is recorded during the operation there will usually be seen a rather marked and rapid rise in pressure following the clamping off of the left carotid artery (Fig. 1). This is simply a matter of central asphyxia and should not be allowed to continue any longer than can be helped. If the injection of the drug into the vertebral artery follows immediately a sudden and profound fall in blood pressure often occurs but is at once succeeded by a return of the pressure almost to the normal. The cause of the sudden fall and rapid rise of pressure is evidently due to the action of the chloroform on the cardio-inhibitory center in the medulla. This appears to be first greatly stimulated and then immediately paralyzed. Following the return of the pressure to near the normal level a second slower





FIG. 1. CAROTID BLOOD PRESSURE TRACING FROM A DOG

The rise in pressure at the beginning of the tracing was caused by asphyxia when the left carotid was clamped. At the highest point in the pressure (H.I.) about 2 cc. of chloroform were injected into the right vertebral artery. The sudden fall following this was due to stimulation of the cardio-inhibitory center in the medulla by the chloroform. This stimulation was rapidly followed by paralysis of the center and the pressure again rose but immediately the vasomotor center was attacked and the pressure rapidly fell to about 55 mm. Hg. Following this both vagi nerves were sectioned, the manipulation of the nerves causing some stimulation as shown by the slowing of the heart. Following this the heart beat became a little faster (indicating the presence of a slight central irritation) and the pressure rose to 90 mm. Hg. at about which level it remained.

progressive and permanent fall ensues. This is due to the paralysis of the vasomotor centers in the medulla oblongata (and possibly the upper end of the cord). Apparently some shock is produced by this action of the chloroform for the pressure usually (but not always) declines to a certain level and then after ten or twenty minutes slowly rises again and maintains this second level permanently. Apparently the distribution and action of the drug in the medulla varies a little in different instances for sometimes the preliminary stimulation of the vagus center for the heart is very marked while in other cases it may be slight or wholly unnoticeable. And the symptoms of a preliminary shock are often wholly absent, apparently indicating that the vital centers have been practically instantly and completely removed from the sphere of action and have thereby failed to produce any secondary or inhibitory effects upon the lower portions of the central nervous system.

It is not always possible to determine exactly how far down the cord below the medulla the chloroform may pass. The vertebral artery sends small branches into the spinal canal from about the sixth cervical vertebra on up to the medulla. These branches may carry blood into the small spinal arteries which pass down almost the whole length of spinal canal. Possibly some little of the drug may sometimes reach the lower cord in this manner. I do not think, however, that this is liable to occur to any marked extent. Probably it may be wholly avoided if the lumen of the vertebral artery is completely occluded by traction on the supporting hook of the aneurism needle for a few seconds before the chloroform is injected and at the same time as the injection is made the blood is allowed to suddenly flow up through the artery. By this procedure the spinal arteries would be filled from the opposite vertebral artery and the pressure in the arteries toward the brain would be materially below normal.

The earliest objection which I anticipated to the method was the passage of the chloroform through the brain capillaries and its return to the heart and lungs. In case this should occur to any considerable extent the animal would probably die. I think this may have actually occurred in two cases for me, but that was early in my trials of the process and as I afterward

recalled I believed that in those instances I had not closed off the jugular veins. Since I have been ligating these veins I have not lost a single animal.

Sherrington states that his "spinal" animals may be kept alive and show good reflexes for as long as ten hours. I have not tried to make any time determinations on the animals upon which I have experimented. In many cases, however, I have made repeated large injections of poisonous drugs into the femoral veins and have kept the animals alive easily for three or four hours. I have no doubt but that with a good animal in which the injection was properly made and care was taken afterward to keep the temperature, etc., in a normal condition, the animal might remain alive for a whole day (and possibly even longer). In my experience the blood pressure, temperature and general vitality have almost universally proved to be of a very much more nearly normal character in animals treated by this method than by the usual processes of mechanically sectioning the cord or destroying the brain. The reasons for this are quite obvious. In a perfectly satisfactory dissection and injection the total loss of blood from the animal need not exceed 3 or 4 cc. and might be even less. A section of the cord is usually accompanied by a greater hemorrhage than this. Again, if care is used in closing off the arteries to the head before the corresponding veins are ligated it may be possible to actually retain more blood in the body of the animal than is present there normally. And further, the operations necessary for a successful injection are vastly less severe and extensive than those necessary for section of the cord.

In no instance have I seen the faintest signs of recovery or return to the normal conditions of the brain in an animal properly injected.

The essential feature of this method consists in a profound chemical destruction of the vital activities of the intra-cranial portions of the central nervous system. A condition is thereby produced which I have been accustomed to refer to as *cephalic coma*. The designation is perhaps inaccurate and poorly selected but not finding a better one at my immediate command, I have preferred to retain it.

From time to time I have considered the possibility of injecting other substances, such as solutions of caffeine, oxalates, cyanides, phenol or formaldehyde. But a more extensive use of chloroform combined with a greater experience in the technique necessary for the process has convinced me that probably no other drug is superior to chloroform for this purpose.

The use of this method may be of much help in many forms of pharmacological and physiological investigation. By it the administration of poisonous or depressing drugs to an animal is reduced to a minimum. The vitality of the animal is proportionately preserved. The brain and medulla are, of course, no longer available for drug reactions. But for all substances which act on peripheral structures the method is immediately serviceable. The animal may be kept perfectly quiescent without the administration of hypnotics or curare. The perverting and confusing influence of these substances, may, therefore, be wholly avoided. It should be duly emphasized, of course, that provision for a thoroughly reliable and permanent artificial respiration which can be properly regulated both as to rate and force must be made before this method can be utilized to advantage.

A question of practical significance is the amount of time required to carry out this process. On the average a good operator, working under perfectly satisfactory conditions, can perform all the essential steps in the method in about ten or twelve minutes from the time the first ether is administered to the animal.

In conclusion it may be emphasized that this action of the chloroform should not be confused with the ordinary anaesthetic action of the substance. For while in its ultimate chemical analysis the two results may really be quite similar, and may possibly differ only in degree, still so far as can be determined at present the end results on the tissues of the brain are widely different. With reference to the rather recent clinical utilization of such substances as hedonal, ether, etc., for the production of general anaesthesia by intravenous injection it is strikingly interesting to note the extremely profound effects which only a few drops of concentrated chloroform may have when injected directly into the arteries leading to the brain and medulla oblongata.

# STUDIES IN ABSORPTION OF DRUGS FROM THE GASTRIC MUCOUS MEMBRANE: I. STRYCHNINE NITRATE

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## INTRODUCTION

No method has yet been employed so far as I have found, to study the absorption of drugs from the gastric mucous membrane, under the normal physiological conditions. Magendie (1) was perhaps one of the earliest to make a special investigation of the absorption of a drug from the stomach. To determine whether strychnine was absorbed from the stomach he isolated the organ by ligating the pyloric and cardiac extremities and injected strychnine into its cavity. Symptoms of strychnine poisoning did not appear until after an hour, from which he concluded that absorption from the gastric mucosa was much slower than from the intestines. His experiments were performed upon the dog.

Colin and Bouley (2) introduced strychnine into the stomach of various animals after ligation of the pylorus or paralysis of the organ by vagotomy. In the normal control dogs, after the administration of 5 to 7 grams of an alcoholic extract of nux vomica, death occurred in fifteen to twenty-five minutes. A dog with the pylorus ligated eighteen hours previous to the administration of the drug, exhibited spasms in fifteen minutes after a dose of 7 grams of alcoholic extract of nux vomica was given, and died in twenty-five minutes. Two dogs in which vagotomy was performed, succumbed, one in ten minutes and the other in twenty-five minutes following the administration of 6 grams of an alco-

holic extract of nux vomica. In another animal in which the vagus nerves were severed and the pylorus ligated, death occurred in eleven minutes after the administration of 5 grams of alcoholic extract of nux vomica. When the drug was injected through the pylorus into the intestine in a similar dose, spasms ensued in twelve minutes and death in twenty-five minutes. To sum up their results on the dog, administration of the alcoholic extract of nux vomica in doses of 5 to 7 grams produced symptoms of poisoning and death as rapidly when the pylorus was ligated or after vagotomy as in the normal animal; and the absorptive power of the isolated stomach was as great as the intestine. These investigators likewise studied the absorption of the extract of nux vomica from the stomachs of other animals. After vagotomy in the rabbit and pig, the administration of an alcoholic extract of nux vomica caused spasms in seventeen and five minutes respectively. In a cow in which the pylorus was ligated, spasms did not occur until four and one-half hours after the injection into the fourth stomach, of 32 grams of the alcoholic extract of nux vomica. In horses, in which the pylorus had been ligated, no symptoms of strychnine poisoning occurred after the administration of 32 grams of the alcoholic extract of nux vomica. In one experiment their observations extended over a period of thirty-five hours. After vagotomy in the horse tremors followed the injection of the drug during the first twenty-four hours, but later passed off and the animal remained otherwise normal. These results on the horse were confirmed by Perosino (3) and his colleagues. Using sulphate of strychnine in doses of 5 grams in aqueous solution, Berard (4) repeated Colin's experiments upon the horse with similar negative results.

Tappeiner (5) using similar methods of experimentation studied the absorption of strychnine from the stomach of the cat. In a normal control cat, weighing 1930 grams, death occurred in eight minutes after the administration of 0.03 gram of strychnine hydrochloride. In two cats in which the pylorus was ligated, death occurred in the first, in one and one-half hours following the administration of 0.05 gram of strychnine hydrochloride in aqueous solution, and in the other cat in three hours following the

administration of 0.1 gram of the same drug. The weight of the first cat was 1300 grams, while the second weighed 1878 grams. With the vagus nerves severed, in a cat weighing 2027 grams, the administration of 0.05 gram of the drug was followed by death in one-half hour. If, instead of aqueous solutions, alcoholic solutions were administered, the results were quite different. With similar doses of the drug in 22.5 and 24.5 per cent solutions of alcohol, death occurred in ten to twenty-five minutes, or equally as rapidly as when the pylorus remained open.

This investigator criticized ligation of the pylorus as being harmful to the stomach and endeavored to avoid this procedure by another method. He introduced into the pylorus, through a fistula in the stomach, a rubber bulb which could be inflated, and thereby occlude the pyloric orifice. His efforts were not attended with success because of vomiting movements which dislodged the rubber bulb. However, he succeeded in obtaining some observations on the absorption of chloral by this method. He found that the aqueous solution was not absorbed while the alcoholic solution of chloral was absorbed as rapidly when the pylorus was occluded as when it was left open. This difference in the absorption of the aqueous and alcoholic solutions of strychnine and chloral led him to suggest that the negative results in the horse as obtained by Colin and Bouley (6) were due to a great dilution of the alcoholic extract of the drug with liquids of the stomach, rather than to a peculiarity in the absorptive power of the stomach of the horse for strychnine. That this is not the case in rabbits has been shown by the experiments of Meltzer (7), who has investigated the absorption of both aqueous and alcoholic solutions from the stomach after ligation of the pylorus. In none of Meltzer's experiments were any symptoms of strychnine poisoning to be observed although his observations extended over hours. His results were quite different from those of Colin and Bouley previously referred to, in which the vagi of the rabbit were severed previous to administering the drug.

In dogs under ether anesthesia in which the cardia and pylorus had been ligated Meltzer (8) found that a dose of 3 mg. of strychnine in aqueous solution, per kilogram of body weight, failed to

produce any symptoms of poisoning in two hours. The normal control dogs were thrown into convulsions by 1.5 mg. of the poison in twenty-five minutes after its administration.

With a much larger dose, in the isolated stomach, 25 mg. of strychnine per kilogram of body weight, in 25 per cent alcohol, spasms were obtained in forty-five minutes, and in this he points out a difference from the results obtained in rabbits. It seems, then, that the addition of alcohol to a solution of strychnine favors its absorption from the isolated stomach of the dog as well as in the cat. From his experiments which included other portions of the alimentary canal, Meltzer concluded that "The absorption of strychnine in the stomach is incomparably lower than in any other section of the alimentary canal," etc. (9). Dixon (10) gives a table showing the relative rate of absorption, in the cat, of strychnine from portions of the alimentary canal isolated by means of ligatures. For the stomach, the time of appearance of symptoms of poisoning is given as thirty minutes.

To sum up the results of previous investigators, two methods have been used to study the absorption of strychnine from the stomach, namely, ligation of the pyloric and cardiac orifices (one or both); and paralysis of the organ by vagotomy. The absorptive power of the stomach differed in different species; also in different observations on the same species. The two methods, i.e., vagotomy and isolation by ligatures have given different results in the same species. In some animals, for example the cat, alcoholic solutions were much more rapidly absorbed than aqueous solutions, but in others this was not the case, notably the rabbit. The dog seems to present the same differences as does the cat in this respect. Great differences exist in the results upon the dog. Some experimenters give results indicating an absorptive power for the stomach equal to that of the intestine, while others give results indicating a very feeble absorptive power. But when these results are analyzed, it is seen that the former were obtained with alcoholic solutions, while the latter were obtained with aqueous solutions of the drug.

All of the foregoing methods have involved procedures which place the stomach in an abnormal condition; and the results of



these procedures have not been adequately controlled. Many of the experiments have been performed under anesthesia, introducing a foreign if not an objectionable factor. Ligating the pyloric and cardiac ends of the stomach or cutting the vagus nerves are in themselves procedures which may at once alter the physiological condition of the organ. In the case of the former there is undoubtedly some alteration of the blood and lymphatic flow, and in addition to this, the injury to the viscus and its nerves produced by the ligatures, must be considered. The method of vagotomy is open to two objections. In addition to the production of an abnormal condition in the stomach, it is difficult, as we know especially from the work of Cannon (11), to be sure of the amount of paralysis which is produced. And even though the organ were paralyzed, it would be difficult to be sure that in all cases the passive movements of the stomach induced by intra-abdominal changes of pressure would not be sufficient to distribute some of the drug to other portions of the gut—not to speak of the capillary movements of the liquid.

Realizing these objections, Brooks in 1907, in some unpublished work (12), endeavored to obtain results free from such criticism. His experiments were performed upon rabbits. To limit the strychnine to the cavity of the stomach and still avoid the procedure of ligating the pyloric extremity, he introduced through an incision into the duodenum a plug of cotton. This plug was pushed up as far as the pyloric orifice of the stomach and was large enough to occlude the lumen. It was previously prepared with oil to prevent absorption, by the cotton, of the strychnine which was in aqueous solution. His results upon the absorption of strychnine were in agreement with those of Meltzer, i.e., no symptoms of strychnine poisoning occurred, but as he felt that his procedure had not been free from objection, he did not publish them.

The results which are herein reported are free from the objections apparent in the foregoing methods.

## METHOD AND RESULTS

The method employed was to introduce the drug into a "miniature" Pawlow stomach. The anatomy of the "miniature" stomach is such that a substance may be introduced into its cavity and brought in contact with its mucosa without entering the remainder of the stomach. The substance is therefore limited to gastric mucosa. Pawlow (13) has shown that the "miniature" stomach furnishes a correct picture of what is taking place in the remainder of the organ so far as secretion is concerned. It seemed then, that it might likewise give a correct picture of the absorptive power of the stomach as a whole. And it should also give a correct picture of excretion from that organ, and of the reflex for vomiting.

On January 26, 1912, a "miniature" stomach was made according to the method of Pawlow (14), in an adult female fox terrier in a moderate state of nutrition. The wound was dressed daily. Sterile vaseline was applied to the wound and adjacent skin to protect it from the action of the gastric juice which escaped from the pouch. The animal quickly recovered from the operation, and during the following three months her appetite was good and she gained in weight. Three weeks after the operation, clear gastric juice which dripped freely from the pouch, was collected and studied. The exact acidity was not determined at this time but on July 1 and 2, 1912, specimens were obtained for this purpose. The acidity on July 1 was 0.32 per cent and on July 2 was 0.35 per cent in terms of HCl. The juice possessed a strong proteolytic property. No evidence of communication between the cavity of the pouch and the stomach could be demonstrated.

At the time the experiments upon absorption were begun the weight of the animal was 8.8 kg. The stomach-pouch was 3 inches deep and would easily permit the entrance of a tube the size of a lead pencil. The mucous membrane at the mouth and that exposed by opening the mouth of the pouch appeared pink and healthy. The pouch underwent peristaltic contractions and secreted clear gastric juice after meals. As further evidence of its intact nerve supply, six minutes after the introduction of a

concentrated solution of chloral hydrate, the animal vomited violently—which was evidently reflex. No symptoms of chloral absorption were manifested. The solution was immediately removed.

There was always a certain amount of erosion of the skin around the mouth of the pouch. This was washed daily and coated with vaseline. Although the eroded area was small when the experiments upon absorption were performed, it was thought necessary to exercise the utmost caution to prevent the solution, which was being studied, from escaping from the stomach-pouch to this adjacent eroded area. The experiments were, therefore, usually performed in the forenoon before the animal was fed, and when the pouch was usually quiet, or relatively so. The animal was placed upon her side or back, in which positions she became accustomed to remain quiet without being restrained, for long periods.

The region around the pouch was cleaned and thickly coated with vaseline. The dose of the drug was measured out into a porcelain dish. A cotton wad was then prepared with a thread attached to one end, and was of such a size that it was saturated by the dose of the drug. While the mouth of the pouch was held open by suitable instruments (avoiding injury), the saturated cotton wad was inserted deeply into the stomach-pouch. In case any of the solution escaped, care was taken to quickly sponge it up as soon as it made its appearance at the mouth of the pouch, and before it escaped to the adjacent surface. In the case of strychnine, the absorption was determined by the appearance of changes in the reflexes—spasms, etc.

The absorption of strychnine nitrate in aqueous and alcoholic solutions was studied and the results are given in six protocols which follow. In experiments no. 1, no. 2 and no. 6, the absorption of aqueous solutions was studied, while in experiments no. 3, no. 4 and no. 5, the solutions were alcoholic. All solutions contained 1.0 per cent of the drug, except in experiment no. 1, in which a 0.5 per cent solution was used. The alcoholic solution contained 20 per cent alcohol, excepting experiment no. 2, which was 10 per cent. In experiment no. 1, the cotton wad containing the strychnine was not deeply inserted into the pouch and was

expelled by a strong peristaltic contraction which terminated the experiment. It is conclusive as far as it was carried.

*Experiment No. 1.* May 9, 1912. Absorption of 0.5 per cent aqueous solution of strychnine nitrate.

Animal is in fine condition. The mucous membrane of the pouch appears pink and healthy. She was fed at about 2 p.m.

- 2.46 p.m. Inserted cotton pledget containing 1 cc. 0.5 per cent strychnine nitrate in distilled water. Three or four drops escaped and were immediately mopped up. The cotton wad was not inserted more than 1 inch. The stomach-pouch is undergoing contractions.
- 2.52 p.m. Powerful contraction almost forced the cotton wad from the stomach-pouch. It was replaced. Approximately 0.5 cc. of clear fluid escaped.
- 2.55 p.m. The reflexes are slightly increased.
- 2.56 p.m. More fluid was forced from the pouch.
- 2.56½ p.m. More fluid was forced from the pouch.
- 3.03 p.m. The reflexes are greatly exaggerated, but do not cross.
- 3.12 p.m. The reflexes are still further increased, but do not cross. Animal is restless.
- 3.32 p.m. The plug was forced out of the pouch by a strong peristaltic contraction. The reflexes are about the same as at last observation.

The first symptoms (heightened reflexes) indicating the absorption of strychnine occurred in nine minutes. A considerable quantity of the drug was lost owing to the unquiet condition of the pouch. In experiments no. 2 and no. 6, the strychnine was retained in the pouch and absorption continued until the onset of convulsions.

*Experiment No. 2.* May 10, 1912. Absorption of 1.0 per cent aqueous solution of strychnine nitrate.

- 12.30 p.m. Animal is in fine condition. She has not been fed today. She was placed on her side, where she remained quietly. The normal condition of the reflexes was studied. Powerful contractions of the pouch occasionally occur.

- 12.34½ p.m. Inserted deeply into the stomach-pouch a cotton wad containing 0.8 cc. 1 per cent strychnine nitrate in distilled water. No fluid escaped.
- 12.55 p.m. No change in the reflexes has occurred up to the present time. Pouch has undergone only comparatively mild peristaltic contractions and no fluid has been expelled.
- 12.56 p.m. A very slight increase in the reflexes is noticeable.
- 1.04 p.m. The reflexes are further increased.
- 1.07 p.m. The reflexes continue to increase. This condition prevails over the entire body.
- 1.26 p.m. Spasmodic momentary extension of both hind legs occurred.
- 1.28 p.m. No fluid has escaped from the stomach-pouch up to the present time.
- 1.35 p.m. *Animal is in a general strychnine spasm.* Her face appears terrified and she apparently tries to control the spasm.
- 1.37 p.m. Animal is quiet. The wad of cotton was removed and the pouch was washed out with a tannic acid solution, which contained a white precipitate as it came from the pouch.
- 1.38 p.m. Animal tried to arise and went into another general spasm.
- 1.45 p.m. Placed animal on floor. She was quite stiff, hyperesthetic and anxious, but was able to walk. She gave indications of a desire to be held. After a short time she was placed in kennel.

*Experiment No. 6.* May 23, 1912. Absorption of 1.0 per cent aqueous solution of strychnine nitrate.

- 12.20 p.m. Animal is in excellent condition. She was placed upon her side and the skin adjacent to the stomach-pouch was cleaned and coated with vaseline. The mucous membrane of the pouch appears healthy. She has not been fed. The pouch occasionally undergoes a feeble contraction. The reflexes were tested and seem to be slightly more sensitive than usual.
- 12.23 p.m. Inserted deeply into the stomach-pouch a cotton wad containing 0.9 cc. of 1.0 per cent strychnine nitrate in distilled water. About 0.2 cc. of fluid escaped from the cotton wad.
- 12.32 p.m. No noticeable increase in the reflexes has occurred.
- 12.35 p.m. The reflexes are now slightly increased.
- 12.37 p.m. Peristaltic contractions of the pouch occur occasionally.

- 12.41 p.m. A marked increase in the reflex irritability is now present.  
12.43 p.m. The muscular tonus is increased.  
12.48 p.m. The reflex irritability is continuing to increase. Mucous is escaping from the mouth of the pouch.  
12.51 p.m. *Animal is in spasms.* The body is rigid and in opisthotonos and the face is drawn. The plug was removed and the stomach-pouch washed out.  
12.53 p.m. Another violent spasm occurred.  
12.55 p.m. The dog was placed upon the floor and jumped about as though on needles—very hypersensitive.  
1.00 p.m. She now runs about and is gradually improving, but the muscles of her legs are stiff and the reflexes are greatly increased.  
1.05 p.m. A large quantity of urine was passed.

The first symptoms indicating the absorption of strychnine, in experiments no. 2 and no. 6, occurred in twenty-one and one-half, and twelve minutes, respectively. The average time for the three experiments was fourteen minutes. Spasms appeared in one hour in experiment no. 2, and in twenty-eight minutes in experiment no. 6. The time of onset of spasms may have been shortened in experiment no. 6, as a result of the administration of strychnine on the two preceding days.

In the following three experiments the absorption of alcoholic solutions of strychnine nitrate was studied. The alcoholic solutions were prepared by mixing distilled water and the required amount of chemically pure alcohol and dissolving the strychnine nitrate in the mixture.

*Experiment No. 3.* May 17, 1912. Absorption of strychnine nitrate 0.9 per cent in alcohol 10 per cent.

- 2.33 p.m. Animal is in excellent condition. The gastric mucous membrane appears normal. The dog was fed a short time previous and the pouch is undergoing peristaltic movements and expelling juice. The reflexes were studied.  
2.45 p.m. Inserted deeply into the stomach-pouch a cotton pledget containing 0.9 cc. of 0.9 per cent strychnine nitrate in 10 per cent alcohol. About 0.2 cc. of juice escaped. This was quickly mopped up with cotton. The pouch is now quiet.

- 2.47 p.m. Pouch is contracting slightly.  
2.57 p.m. The reflexes up to the present time show no increase.  
3.02 p.m. The reflexes are now slightly increased.  
3.08 p.m. Reflexes further increased. Animal can inhibit them.  
3.14 p.m. Hind legs twitch.  
3.29 p.m. Reflexes are now greatly increased but do not cross.  
3.40 p.m. Reflexes cross in fore-legs. The muscular tonus is greatly increased.  
3.45 p.m. Face is anxious—respiration panting—neck is stiff and head is drawn backward.  
3.51 p.m. Animal is less anxious and hyperesthesia has decreased.  
3.59 p.m. The action of the strychnine seems to be passing off. The cotton wad was removed from the stomach-pouch. Upon removing the cotton from the pouch, about 1 cc. of fluid escaped, which was collected in a clean receptacle. Some of this fluid was injected into the dorsal lymph sac of a frog. Tetanus followed the injection in the frog in two minutes.

*Experiment No. 4.* May 21, 1912. Absorption of strychnine nitrate 1.0 per cent in alcohol 20 per cent.

- 1.43 p.m. Animal in fine condition. Pouch occasionally contracts moderately, and a small amount of fluid is expelled. The gastric mucosa appears normal. Respiration, 15. Pulse, 102.  
1.46 p.m. A cotton pledget saturated with 0.9 cc. of 1.0 per cent solution of strychnine nitrate in 20 per cent alcohol was inserted into the "miniature stomach." About two drops escaped which was mopped up with cotton. The pouch occasionally contracts feebly, but no fluid escapes.  
1.54 p.m. The reflexes are now slightly increased.  
1.58 p.m. Pulse, 84.  
1.59 p.m. The reflexes are further increased.  
2.00 p.m. The stomach-pouch is now contracting strongly. A drop of fluid escaped which was removed.  
2.01 p.m. Another drop of fluid escaped.  
2.02 p.m. The reflexes are further increased, but are limited to the area stimulated.  
2.09 p.m. The reflexes cross. The left fore-leg is stiff.  
2.11 p.m. An occasional violent twitching of all four legs occurs. A tap on the nose with a forceps causes a jerk of the muscles of the entire body.

- 2.22 p.m. The stomach-pouch is contracting powerfully but no fluid is escaping.
- 2.45 p.m. Animal is very hyperesthetic.
- 2.54 p.m. Animal has become more quiet.
- 2.54½ p.m. A momentary spasmodic jerk of the fore-limbs occurred.
- 2.55 p.m. Animal is in violent tremors.
- 2.58 p.m. Animal is becoming quiet.
- 3.00 p.m. The pouch has been quiet for some time but began contracting at this time.
- 3.03 p.m. The pouch is quiet again.
- 3.15 p.m. Pulse, 90.
- 3.20 p.m. The animal is quiet but very hyperesthetic.
- 3.25 p.m. Respiration, 15.
- 3.36 p.m. The animal remains quiet and hyperesthetic. Cotton pledget was removed and the pouch was washed out.

*Experiment No. 5.* May 22, 1912. Absorption of strychnine nitrate 1 per cent in alcohol 20 per cent.

- 1.39 p.m. Animal is in fine condition. She has not been fed today. The stomach-pouch is quiet. The reflexes were studied and it seems that they are slightly more sensitive than usual.
- 1.49 p.m. A cotton pledget containing 0.9 cc. 1 per cent strychnine nitrate in 20 per cent alcohol was inserted into the miniature stomach-pouch. About 0.1 cc. of fluid escaped.
- 1.54 p.m. A drop of fluid escaped from the pouch.
- 2.01 p.m. The reflexes now show the first increase.
- 2.08½ p.m. The pouch has remained quiet up to this time.
- 2.10 p.m. The reflexes are further increased but do not cross.
- 2.12 p.m. Another drop of fluid escaped from the pouch.
- 2.17 p.m. The tone of the muscles is increased. The fore-legs are stiffened out.
- 2.21½ p.m. The reflexes remain the same.
- 2.24 p.m. Another drop of fluid escaped.
- 2.27 p.m. The pouch still remains quiet.
- 2.35 p.m. Tremors are present in the muscles of the face. The respirations are slightly increased in depth.
- 2.37 p.m. The fore-legs are again stiffened out. Another drop of fluid escaped from the pouch.
- 2.38 p.m. The fore-legs are now relaxed.



- 2.57 p.m. Some fluid escaped from the pouch during spasmodic respirations. The reflexes remain increased as before but do not cross.
- 3.01 p.m. The animal was placed upon her feet. In doing so, approximately  $2\frac{1}{2}$  cc. of fluid spurted out, which was caught in a clean porcelain dish prepared for the purpose. The cotton pledget was removed. This fluid was tested for strychnine by making several injections into frogs. It was found that the equivalent of 0.75 minims of the undiluted fluid was sufficient to produce spasms in a frog. This indicated that a large quantity of the strychnine had not been absorbed from the stomach-pouch.

Based upon the preceding protocols the following table has been constructed to facilitate a ready comparison of the results.

*Table showing results of experiments on absorption of strychnine from the stomach*

NUMBER OF EXPERIMENT	DATE	DOSE OF STRYCHNINE	KIND OF SOLUTION	TIME OF ADMINISTRATION	FIRST INCREASE IN THE REFLEXES		SPASMS	
					Time of Appearance	Time required for Appearance	Time of Appearance	Time required for Appearance
1	5/ 9/12	1 cc. 0.5%	Aqueous	2.46	2.55	9 min.	See reference(17)	
2	5/10/12	0.8 cc 1.0%	Aqueous	12.34½	12.56	21½ min.	1.35	60½ min.
6	5/23/12	0.7 cc 1.0%	Aqueous	12.23	12.35	12 min.	12.51	28 min.
3	5/17/12	0.7 cc. 0.9%	Alcoholic 10%	2.45	3.02	17 min.	None at 3.59	None in 74 min.
4	5/21/12	0.9 cc. 1.0%	Alcoholic 20%	1.46	1.54	8 min.	None at 3.36	None in 110 min.
5	5/22/12	0.8 cc 1.0%	Alcoholic 20%	1.49	2.01	12 min.	None at 3.01	None in 72 min.

#### DISCUSSION

In these experiments certain features stand out prominently. First, as regards the time required for the first appearance of symptoms of strychnine absorption. The average time of the

appearance of such symptoms was about fourteen minutes for the aqueous as well as the alcoholic solutions. The shortest time was eight minutes (experiment no. 4). These results were obtained with doses of about 1 mg. per kilogram of body weight which was only one-third the dose used in Meltzer's (15) experiments in which no symptoms of absorption occurred. If the results obtained from the Pawlow miniature stomach may be considered applicable to the normal (entire) stomach, the conclusion must follow that the stomach of the dog possesses a greater power for absorbing strychnine than has been indicated by previous experiments, excepting those of Colin and Bouley. But in their experiments the cardia remained open. Meltzer (16) has stated that under such conditions his results were not "clear" for the animals frequently vomited, bringing the drug into the oesophagus and pharynx which he has shown to possess greater power for absorbing strychnine than the isolated stomach. Furthermore, in their experiments alcoholic solutions were used, and there is an agreement that alcoholic solutions of strychnine are absorbed with greater rapidity than aqueous solutions from the stomach of the dog which has been isolated with ligatures. From the Pawlow miniature stomach, this is not the case. The addition of alcohol to the solution containing strychnine retards instead of hastening the absorption of the drug. The result is most strikingly shown in experiment no. 5. In this experiment, after one hour and twelve minutes following the administration of the alcoholic solution of strychnine, the symptoms of strychnine poisoning did not seem to be increasing, so the experiment was concluded. However, it was found that the pouch contained more fluid than was originally administered and that this fluid contained a large percentage of the original dose of strychnine. Whereas 0.9 cc. of an alcoholic solution of strychnine was introduced into the stomach-pouch, about 2.5 cc. of liquid was recovered, not including that which was absorbed in the cotton pledget. Less than an equivalent of one minim of this liquid was sufficient to produce spasms in a small frog. In three experiments with alcoholic solutions of strychnine, absorption never proceeded to the point of producing spasms, while in two experiments with

aqueous solutions, spasms occurred in both cases (17). In view of these results it would seem that the method of isolating the stomach by ligatures, in studying its power of absorption, is open to serious objection.

In view of the slow elimination of strychnine, it might be supposed that in two or more experiments performed on succeeding days, the onset of strychnine poisoning might be more readily obtained in the second, or third experiment. Experiment no. 6 was one of three experiments performed on consecutive days, and it is possible that this may account for the fact that spasms occurred in half the time required for their appearance in experiment no. 2. In experiments no. 5 and no. 2, the conditions as regards previous experiments were comparable. They were both performed on days following another experiment. Comparing the results, it is found that in experiment no. 2, in which an aqueous solution was used spasms occurred in one hour, while in experiment no. 5, in which an alcoholic solution was used, spasms did not occur in an hour and twelve minutes, and that a considerable quantity of the strychnine administered, remained unabsorbed in the stomach-pouch.

Since the same animal was used throughout these experiments the factor of individual variation was eliminated. The comparative value of the results should therefore be increased. In considering the absorptive power of the entire stomach it must be borne in mind that these results indicate the absorption of strychnine from a relatively small area of gastric mucous membrane.

#### CONCLUSIONS

1. Strychnine nitrate in aqueous and alcoholic solutions is absorbed by the mucous membrane of the Pawlow "miniature stomach" of the dog.

2. The rapidity of absorption of strychnine from the gastric mucous membrane, as determined by the Pawlow miniature stomach, is greater than is indicated by previous experiments based on other methods.

3. Strychnine nitrate in alcoholic solutions (10 and 20 per cent alcohol) is not absorbed as readily as in aqueous solution. The first appearance of symptoms indicating the absorption of the drug occur in about the same time in both classes of solutions. The later symptoms—spasms—could not be induced in a similar period of time with a quantity of the drug in alcoholic solution, which caused spasms when administered in aqueous solution, although the reflexes became greatly increased.

4. Ligation of the pyloric and cardiac extremities of the stomach seems to alter its normal power for absorption.

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- (17) In the first experiment with an aqueous solution the experiment was prematurely brought to a close through an accident.

## THE PULMONARY ACTION OF THE ADRENAL GLANDS

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In another article<sup>1</sup> I have described a method for destroying the brain and medulla of an animal for experimental purposes. The spinal animal thus produced at once becomes an excellent subject for the study of peripheral pulmonary activities. The method which I have used is as follows. In the fifth or sixth intercostal space on the right side, about  $\frac{1}{2}$  inch from the outer border of the sternum an incision is made in the skin and fascia and then a scalpel is passed into the thoracic cavity. The blade should enter the chest just above the rib in order to avoid the vessels which run below the ribs. With the hooks of two aneurism needles the opening thus made is enlarged, great care being taken to see that the parietal pleura is well opened up. Next a glass tube about  $\frac{3}{8}$  inch in diameter and having about 1 inch at one end bent at a right angle is inserted in the opening in such a manner that the short (bent) arm lies closely against the pleura of the chest cavity inside. With a haemostat the edges of the wound are then brought tightly together around the long arm of the glass tube. A cork with a hole in it may be slipped down over the projecting tube and thus help to hold it in place. On the other side of the chest a similar tube is inserted and may be left open. Or it may be closed when desired with a piece of rubber tubing and a clip. The second tube is inserted to equalize the pressure on each lung. The anterior mediastinum must not be punctured. A rubber tube leads from one of the glass tubes to the recording instrument. A large tambour with a deep cup

<sup>1</sup> Jour. of Pharm. and Exper. Therap., vol. iv, no. 1, 1912.

may be used very well. A Brodie bellows is perhaps, more satisfactory. By this means the volume changes of the lungs can be quite readily recorded on the drum. Incidentally it might be suggested that this method is excellently adapted for ordinary student experiments in pharmacology. It may be used also for demonstrating many physiological actions in connection with the lungs.

It is necessary to make a few suggestions regarding certain objections which may be offered concerning this method. It might be suspected that the changes occurring in the circulation would interfere with the movements of the lungs. That such is not the case has already been fully proven for plethysmographic methods by Dixon and Brodie.<sup>2</sup> And I have assured myself that the conclusions formed by them may be equally well applied to this method. So long as the nerves or muscles of the lungs (bronchioles) themselves are not affected great variations in the blood pressure either in the nature of a rise above or a fall below the normal have practically no influence whatever on the relative amplitude of the expansion movements of the lungs under the artificial respiration. As a test of this point a single example may be mentioned. If the abdominal aorta be dissected out and an adjustable clamp be placed on it in such a manner that the vessel can be occluded or opened at any desired time, then it is found that if, while a regular lung-volume tracing is being recorded, the aorta be closed off and the pressure above the diaphragm be thereby more than doubled, as seen by the record from the mercury manometer, practically no change whatever will be produced in the extent of the lung excursions (Fig. 1). If the clamp on the vessel be operated by hand then probably some disturbance will be produced, either in the position of the abdominal viscera or of the crura of the diaphragm, which may show on the tracings. The mechanical nature of these can be recognized immediately and confusion will not thereby be pro-

<sup>2</sup> Dixon and Brodie: *Jour. of Physiol.*, 1903, xxix, p. 97. These authors give a brief historical description of most of the methods previously used for recording pulmonary changes (bronchial). See also Trendelenburg: *Zent. f. Physiol.*, 1912, xxvi, p. 1.

duced. And further, when the nerves to the bronchioles have been fully paralyzed, the marked rise in blood-pressure produced by the intravenous injection of epinephrine do not cause any

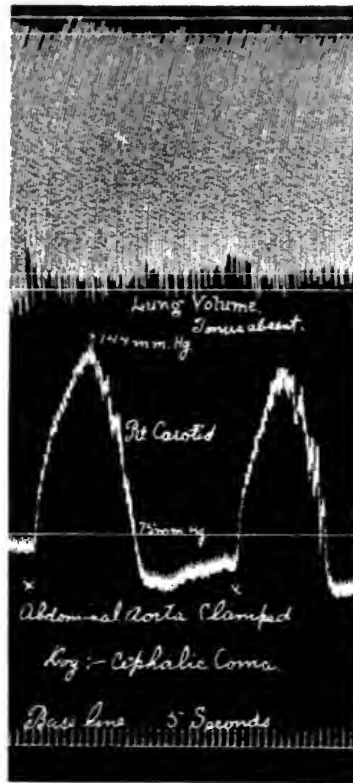


Fig. 1. LUNG VOLUME AND CAROTID BLOOD-PRESSURE TRACINGS FROM A DOG. BRAIN AND MEDULLA DESTROYED BY CHLOROFORM

At the points indicated the abdominal aorta was clamped by a specially devised instrument. The blood pressure at once rose to about twice its former height. But little change, however, was produced in the extent of the respiratory excursions. It will be noted that the upper and lower limiting lines varied a little. This was due not to the blood pressure but to the slight mechanical disturbance of the diaphragm and abdominal viscera by the gradual opening and closing of the aortic clamp with the hand.

changes in the regular amplitude of the volume record. This method, of course, is not suited for the study of the lung-volume changes under convulsive poisons. For any spontaneous move-

ments of the chest walls or diaphragm will at once affect the record. The animal which is successfully injected with chloroform, however, will not cause this disturbance unless affected by some marked extraneous influence.

Certain precautions are necessary for the successful use of this method. The volume and rate of the insufflated air must be regular and properly adjusted. Usually a medium-sized expansion is best to bring out the pulmonary reactions. It appears that the vagus endings in the bronchial muscles are extremely susceptible to abnormal influences. Among these may be mentioned fatigue from electrical excitation of the vagus trunk. It often occurs that a very short vagus stimulation may so influence the nerve endings, or some other structures, that later stimulation of the nerve trunk may have no perceptible effect at all on the bronchioles. Generally the pulmonary response to drugs is more easily and certainly elicited than is that to vagus stimulation. By the use of this method for non-convulsant drugs, the administration of curare to the animal may be avoided, and thus the obscuring actions of the latter substance are not present. In cases where curare must be used the method is still desirable for no ether to allay the pain of the animal is required. Special objections must be offered to the administration of the volatile anaesthetics for Dixon and Brodie have shown that these substances may themselves paralyze the nerve endings in the lungs. And most hypnotics appear to be also open to similar objections. This method is much to be preferred to those procedures in which the chest is opened and the lungs are manipulated in what must be in any case a rather severe manner for such delicate structures as the lung tissues and their entering nerves and blood and lymph vessels. And the total loss of not more than a very few cubic centimetres of blood from the animal during the whole experiment is a favorable feature. In a careful and successful operation it is possible that the total hemorrhage may not exceed 3 or 4 cc. and might be less.

In the course of a series of experiments performed by Grossmann<sup>3</sup> in 1887 on the production of pulmonary edema by muscar-

<sup>3</sup> Grossmann, M.: *Zeit. f. klin. Med.*, 1887, xii, p. 579.



ine he accidentally discovered that the restriction of the air movements in the lungs of animals treated with muscarine might be annulled by stimulating the cardiac accelerator nerves. He was inclined to attribute this result to vascular changes. Brodie and Dixon (1903) previous to having read Grossmann's paper stimulated the nerve fibers leaving the ganglion stellatum with completely negative results. But when they came to read Grossmann's article they decided that the most probable explanation was that some bronchodilator fibers passed from the ganglion into the lungs. They accordingly repeated their former experiments with absolutely negative results. They, therefore, concluded that no dilator fibers to the bronchial muscles pass through the ganglion stellatum.

In 1911 Januschke and Pollak<sup>4</sup> published an article on the pharmacology of the bronchial musculature. These authors demonstrated that the intravenous injection of epinephrine into an animal in which a bronchial constriction has been produced by the previous injection of muscarine causes a dilatation of the bronchioles. Previous to having read their article I had performed a long series of experiments and had arrived at conclusions quite similar to those to which these authors came. I had, however, used for the most part entirely different methods to prove that my observations were correct. This finding is of such immediate and marked clinical and biological importance that I should like to at least state that I have been able to fully verify practically all of the actual observations which Januschke and Pollak made. This, however, applies only to their experimental results. Their theoretical conclusions I have not been able to verify. Since epinephrine produced a dilatation of the bronchioles these authors concluded that sympathetic fibres probably passed up beyond the stellate ganglion and in some way communicated with the vagi nerves, for it has long been known that under proper experimental conditions the presence of both broncho-constrictor and broncho-dilator fibres can be shown in the vagus nerves.

<sup>4</sup> Januschke, H und Pollak, L.: Arch. f. exper. Pathol. u. Pharm., 1911, lxvi, p. 205.

In the earlier experiments of this series I arranged the animals as I have described above for recording the lung volume changes and right carotid pressure. The animal (dog) was then turned over a little on to its left side and an opening was made in the right thoracic wall. By an extrapleural operation the right sympathetic nerves were picked up and isolated for a distance of 1 or 2 inches. A thread was then tied to the nerve trunk which was at once divided below the thread. This left the cephalic end of the nerve attached to the thread which was passed through two small holes which were blown in the opposite sides of a small flat-bottomed, straight-sided vial. These holes were about  $\frac{1}{4}$  inch from the bottom of the vial and when the full length of the thread was pulled through them the vial was placed down into the wound and the nerve trunk was drawn inside the vial which was then slipped well up toward the apex of the chest cavity. The obstructing rami communicantes were sectioned in order to free the nerve trunk. The points of a platinum electrode were then passed down inside the vial and placed on the nerve trunk. All the apparatus was then carefully adjusted and a small injection of pilocarpine given. The dose was purposely small in order to avoid producing too strong a tonic action on the bronchial constrictor nerve endings. When the broncho-constriction had become fully developed the current was turned on and the nerve trunk was stimulated. No influence whatever on the lung volume was observed. It should be noted that it is necessary for the bronchioles to be somewhat constricted before any agent which dilates them can give any visible results. Consequently some constricting drug must be given. Pilocarpine is the most accessible and effective substance which is usually at hand.

My results in these experiments appear to coincide exactly with the earlier observations of Dixon and Brodie, that the sympathetic trunk carries no bronchodilator fibers.

Following these experiments I took up a different line of experimentation. In these later experiments the extrapleural operation for the isolation of the sympathetic trunk was performed at a lower level and the sympathetic chain was picked up a short distance above the diaphragm. The chain was again ligated,

divided and drawn through the holes in the bottom of the vial. The vial was then moved down as low as possible behind (above) the diaphragm. The apparatus was carefully adjusted, a small injection of pilocarpine was made into the left femoral vein and the broncho-constriction was recorded. The slowing of the heart and the fall in blood pressure usually preceded the broncho-constriction a little. When the lung volume had reached its maximum decrease and had continued thus for a little time the current was turned on and the sympathetic trunk was stimulated. A fairly abrupt and rather marked rise in blood-pressure followed. The heart beat also soon became somewhat faster and the effects of the pilocarpine gradually disappeared. After a well marked latent period the amplitude of the lung excursion began to increase (Fig. 2). This began some time after the blood pressure had started to rise and the maximum dilatation of the bronchioles was not reached until some time after the maximum blood-pressure had been passed. A little later the amplitude of the lung movements again began to slowly decrease. This decrease was not usually very marked but in all probability represented a slight return to the influence of the pilocarpine whose action is rather prolonged on the bronchostrictor nerve endings. A bronchial dilatation in practically all respects similar to the one thus obtained may be produced by the slow injection of a very minute dose of epinephrine. The slight gradual return of the previous pilocarpine action is an important feature in checking up the results, for it is very characteristic of epinephrine to produce only a transient dilatation which soon disappears as the drug ceases to act.

From the results it seems not unreasonable to conclude that stimulation of the splanchnic nerves causes a secretion of epinephrine which in turn is carried to the lungs and produces a stimulation of the broncho-dilator nerves. And on the basis of these experiments it seems probable that both Grossmann and Dixon and Brodie were correct in their observations. For while in neither instance did these investigators state what method they used for isolating and stimulating the sympathetic nerves, yet it seems quite probable that Grossmann simply picked

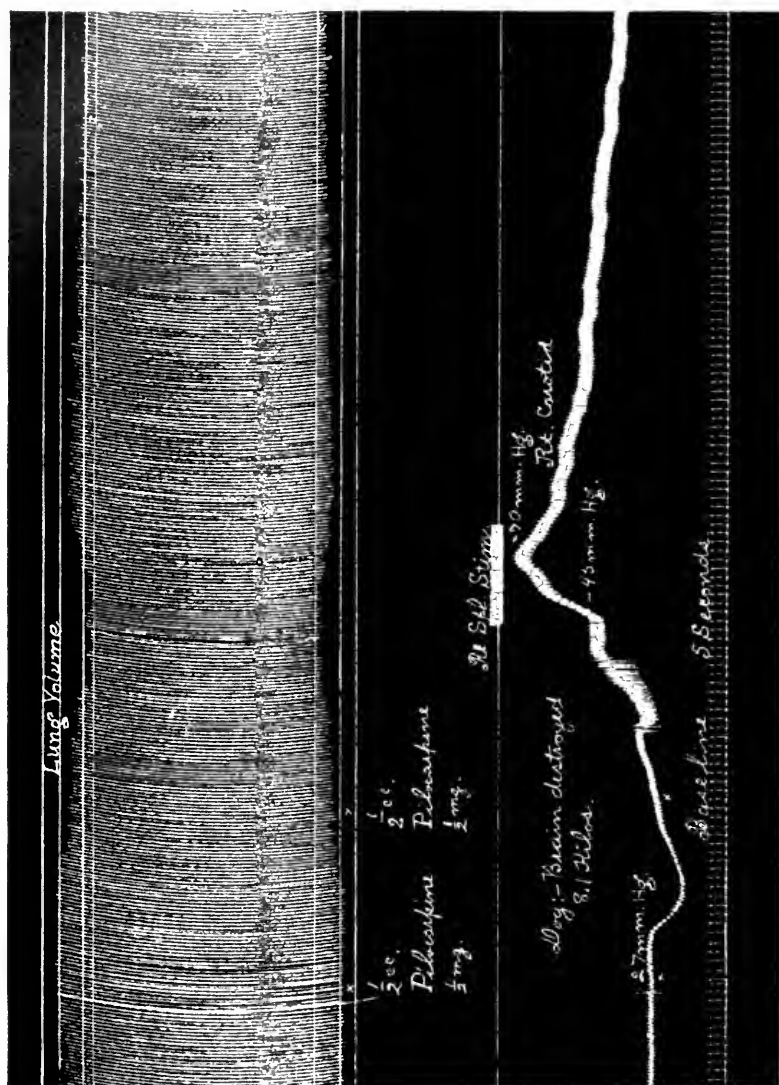


FIG. 2. LUNG VOLUME AND CAROTID BLOOD-PRESSURE TRACINGS FROM A DOG. BRAIN AND MEDULLA DESTROYED BY CHLOROFORM

At the points indicated two injections of 0.5 mg. each of pilocarpine were made into the left femoral vein. A medium bronchoconstriction was thus produced. At the position marked by the signal the right splanchnic nerves were stimulated with the platinum electrodes. The blood-pressure rose and a little later the extent of the lung excursions began to increase. These did not reach their maximum until about forty-five seconds after the blood pressure began to decrease. The sympathetic chain had been sectioned at the level of the tenth rib.

up the accelerator fibres passing from the stellate ganglion to the heart and stimulated them without sectioning the sympathetic trunk below the ganglion. And the well marked latent period which he observed probably represented the time necessary for the stimulation of the sympathetic fibers at the stellate ganglion, either directly or indirectly, to pass off down the sympathetic chain and increase the adrenal secretion. This in turn was carried to the lungs and counteracted the constricting influence of the muscarine as recorded in his observations.

And on the other hand I suspect that Dixon and Brodie divided the sympathetic fibers so that no stimulation could leak off and pass down to the adrenals. It is not impossible, however, that all three of these investigators may have proceeded along exactly similar lines for another very important factor must be considered in such experiments as these. It has been shown by a number of investigators that a large range of influences may cause a secretion of the active principle in the adrenal glands. And it seems quite probable that some of these influences may, to all practical purposes, exhaust the supply of active substance which the glands can secrete at any given time. Among these agencies Cannon<sup>5</sup> has enumerated fear, asphyxia, nicotine,<sup>6</sup> etc., and in all probability many other influences may also produce this result. Some of these will be mentioned below. Thus it may readily be seen that Dixon and Brodie may have experimented on animals in which no very noticeable pulmonary dilatation could have been produced by the quantity of active substance remaining in the glands even if the splanchnic nerves had been stimulated directly. Perhaps Elliott's observation<sup>7</sup> that epinephrine itself may cause a discharge of epinephrine should be considered here.

In their experiments on the bronchial innervation Dixon and Brodie<sup>8</sup> obtained only slight and inconstant results with epi-

<sup>5</sup> Cannon, W. B. and De la Paz, D.: *Am. Jour. of Physiol.*, 1911, xxviii, p. 64. *Ibid* (with Hoskins, R. G.): 1911, xxix, p. 274.

<sup>6</sup> Cannon, W. B., Aub, J. C., and Binger, C. A. L.: *Jour. of Pharm. and Exper. Therap.*, 1912, iii, p. 379.

<sup>7</sup> Elliott: *Jour. of Physiol.*, 1905, xxxii, p. 427.

<sup>8</sup> *Loc. cit.*

nephrine. Similarly with nicotine they sometimes produced slight constriction and at others a little dilatation. With respect to the epinephrine the explanation seems to be fully justified that they failed to administer the drug while the bronchioles were tonically contracted. Otherwise it is difficult to understand why they did not observe the broncho-dilatation. And Cannon's observation that nicotine may cause a secretion of epinephrine seems to explain part of the pulmonary findings with that drug. For the variable double action of nicotine on all the sympathetic ganglia and the accompanying liberation of epinephrine would almost certainly result in variable effects from the nicotine on the bronchioles. It seems that under the most favorable conditions the bronchioles are very sensitive to the dilator action of epinephrine, and if the technique were not so much involved it is possible that this reaction might be used as a test for epinephrine. Special mention should perhaps be made that the rise in blood-pressure which follows stimulation of the splanchnic nerves is not the cause of the broncho-dilatation. For it is quite easy to show that after a small dose of atropine very extensive changes in the general blood-pressure do not affect the regular amplitude of the lung movements. And simple mechanical increase in blood-pressure, such as can be produced by compression of the aorta as mentioned above, does not cause bronchial changes such as follow splanchnic stimulation. And conversely it has been shown by Januschke and Pollak<sup>9</sup> that after the paralysis of the vaso-constrictor receptors lying in the path of the thoracico-lumbar autonomies by ergotoxine then epinephrine still causes a dilatation of the tonically constricted bronchioles although no change in the general arterial pressure may occur.

Dixon and Brodie (1903) after experimenting with commercial "ergotin" and with the "sphacelotoxin" of Jacobi decided that these bodies possessed no action on the bronchioles. At that time the available preparations of ergot were of an extremely questionable character. More recently, however, it has become

<sup>9</sup> Loc. cit., p. 217.

possible to obtain reliable preparations of the active principle of ergot. I suspect the uncertain character of their preparations greatly influenced the results obtained by Dixon and Brodie.

It has been shown by Dale<sup>10</sup> that the active principle of ergot will produce a powerful and prolonged tonic constriction of the cat's pupil when intravenously injected. Atropin will not remove this constriction and after large doses of ergotoxine only the strongest stimulation of the cervical sympathetic will cause any noticeable dilatation. It seems, therefore, that the ergotoxine acts largely on the muscle fibers but that the sympathetics are not completely paralyzed.

A further interesting observation made by Dale and on which he laid but little emphasis was the fact that the intravenous injection of ergotoxine caused a "brief dilatation of the pupil lasting a few seconds only." This was unaffected by section of the cervical sympathetic nerve but was absent after removal of the superior cervical ganglion. He unfortunately did not state whether the ganglion was removed immediately before the ergotoxine was injected or whether the removal had occurred some time previously. The inference, however, is that the excision had preceded the administration of the drug but a short time. He offered no explanation for the brief preliminary dilatation, nor for its disappearance after removal of the ganglion.

When the brain of an animal (dog) is destroyed by chloroform and the lung volume is recorded in the manner above described, then the injection of ergotoxine phosphate (Burrows, Welcome and Company) in *small doses* (1mg.) will give a marked rise in blood-pressure which is shortly followed by a dilatation of the bronchioles. It is necessary that some tonic constriction must have been present in the bronchioles before the injection for the reaction to be demonstrated satisfactorily. The dilatation is of a transient character and soon gives way to rather noticeable constriction. If now large doses of ergotoxine are given no further dilatation occurs but a rather pronounced constriction may follow (Fig. 3). Injection of epinephrine may now again dilate the lungs.

<sup>10</sup> Dale, H. H.: Jour. of Physiol., 1906, xxxiv, p. 186.

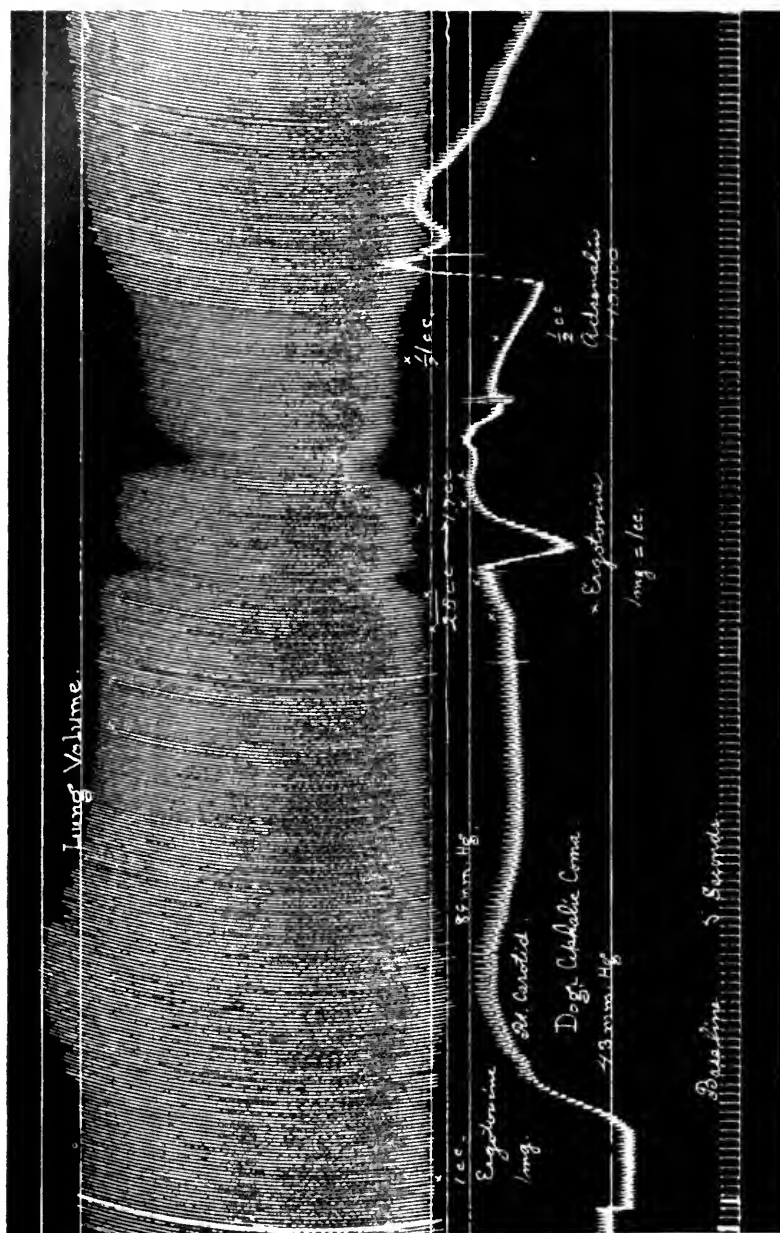


FIG. 3. LUNG VOLUME AND CAROTID TRACINGS FROM A DOG. BRAIN AND MEDULLA DESTROYED

At the point indicated 1 mg. of ergotoxine was injected. The blood-pressure rose and a little later the lung excursion increased. After a time 25 mg. of ergotoxine were injected. These caused some fall in blood-pressure and a pulmonary constriction. Later adrenalin caused a dilatation.



In the light of other observations it seems fair to conclude that the preliminary transient pulmonary dilatation may be due to a stimulation of the sympathetic endings in the adrenal glands. And the failure to obtain the dilatation after one or two injections of ergotoxine also bears out this supposition, for so active a stimulation of the glands as this drug probably produces might very soon exhaust all the readily available supply of the active substance. Probably this action is wholly analogous to and of identically the same origin as the brief preliminary dilation of the pupil observed by Dale. And in his observations I suspect that in simply dividing the cervical sympathetic he did not irritate or in any way stimulate the sympathetic chain below, so that the easily available supply of epinephrine in the glands was not affected. Consequently the stimulation caused by the ergotoxine produced a secretion. Unfortunately he did not state how often such a result (dilation) could be produced by separate injections into the animal. But the prolonged tonic constriction which soon reached its maximum (within ten minutes) would preclude many observations for the mydriasis. And probably when Dale removed the superior cervical ganglion he at the same time stimulated nerve fibers which, either directly or indirectly eventually carried this stimulation to the adrenal gland on the same side. An exhaustion of that gland could conceivably result from such a process. And since strong sensory stimulation and various other factors may also produce adrenal secretion it seems possible that in the more extensive surgical process necessary to remove the superior cervical ganglion some condition may have arisen which caused a partial or complete exhaustion of the adrenal secretion. On this supposition the observation of Joseph and Meltzer<sup>11</sup> that after removal of the superior cervical ganglion the pupillary sympathetic myoneural junctions become more sensitive to adrenalin and after several days may be dilated by splanchnic stimulation would seem to furnish an excellent opportunity to test out the power of ergotoxine to stimulate the adrenal glands.

<sup>11</sup> Joseph, Don R. and Meltzer, S. J.: *Am. Jour. of Physiol.*, 1912, xxix, *Proc. of the Am. Physiol. Soc.*, p. xxxiv.

It is possible that the prolonged tonic contractions of the pupil observed by Dale may have its counterpart in the bronchial musculature. For the drug can undoubtedly produce a definite contraction of the bronchioles. Possibly such an action might be concerned to some extent in the cases of pulmonary gangrene in chronic ergot poisoning. Unfortunately I have not had an opportunity so far to test out the final pulmonary action of the drug. It seems evident, however, that in the lungs the dilator nerve endings continue to exercise a very considerable action which may even reach the normal in its extent and muscarine can still produce the broncho-constriction which epinephrine can remove.

It is important to lay marked emphasis on the pulmonary dilator action of epinephrine. For this drug is usually mentioned as acting specifically on the termination of the sympathetic nerves arising from the lumbar and dorsal regions of the spinal cord. And it is generally accepted as true that no dilator fibers reach the lungs except by way of the vagus nerves. If this supposition is true then epinephrine here stimulates the endings of a cerebral autonomic nerve. Januschke and Pollak attempted to avoid this conclusion by assuming the existence of some communication as being made between the true sympathetic chains and the vagi nerves. If the observations of Dixon and Brodie and of myself are true then no dilator fibers pass from the thoraco-lumbar autonomies to the lungs. And we are thus bound to conclude that epinephrine stimulates the vagus broncho-dilator endings. I have no direct evidence at present that the broncho-constrictor endings are affected by epinephrine. But Dixon and Brodie *occasionally* observed a slight temporary constriction with large injections. They suspected, however, that this was due simply to the great vascular engorgement which was present. I have no doubt but that their conclusion was wholly correct.

Some further observations which I have made on another drug may be of interest here. If in an animal whose brain is destroyed by chloroform and the lung volume is recorded there be injected a sufficient amount of pilocarpine to produce a well-

marked broncho-constriction, then it is found that the injection of hydrochinon will also dilate the bronchioles (Fig. 4). At the same time there is a rise in blood-pressure and the heart tends to become slow. The exact cause of the broncho-dilation in this

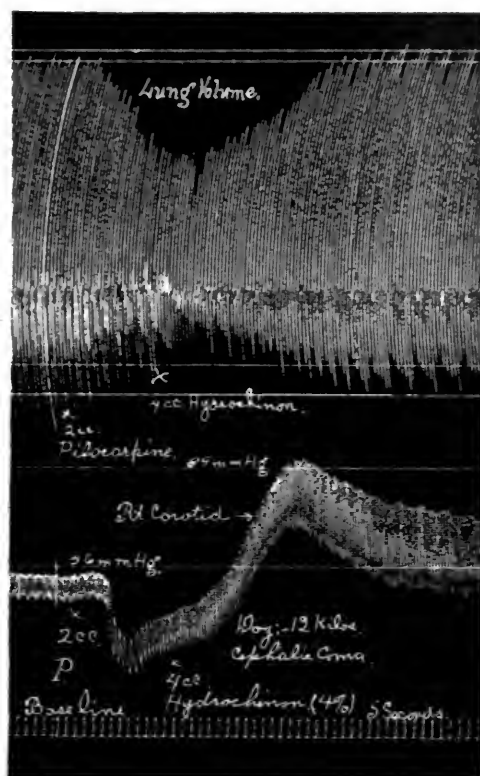


FIG. 4. LUNG VOLUME AND CAROTID TRACINGS FROM A DOG. BRAIN AND MEDULLA DESTROYED

At the point indicated 2 mg. (2 cc.) of pilocarpine were injected. A marked bronchoconstriction was produced. Later 160 mg. (4 cc.) of hydrochinon were injected. This caused a bronchial dilatation.

case is obscure. The marked similarity in chemical structure between this body and epinephrine suggests at once a similar action. It is probable that this may be true to at least some extent. But other features must also be considered. A good

preparation of hydrochinon (Kahlbaum) tends to produce slight convulsive movements in the animal. Since the brain is destroyed these must arise from the cord. They may be due to a direct stimulation of the motor cells by the drug. If this be true it is conceivable that the discharge of nervous impulses thus generated may stimulate the adrenals. But further possibilities exist, for hydrochinon also tends to produce methaemoglobin and a partial asphyxia may thus be produced. Possibly this may stimulate the cord indirectly with a further chance for epinephrine to thus be secreted. It seems hardly likely that the drug should depress the broncho-constrictor nerve endings.

In conclusion it may be stated that the total results of these experiments bear out the opinion that in the intact animal one of the functions of the adrenal glands is to assist by means of their internal secretion in counteracting pathological processes or products which tend to produce an abnormal broncho-constriction.

## A METHOD OF STANDARDISING PITUITARY (INFUNDIBULAR) EXTRACTS

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During recent years the watery extract or decoction of the posterior or infundibular lobe of the pituitary body has acquired wide use and importance in therapeutics, and seems likely to take its place among drugs of recognised and definite efficiency. The knowledge concerning the chemical nature of its active principle (or principles) is of the scantiest description, and there seems no prospect at present of the control of its efficiency by chemical methods. At the same time what is known indicates that the principle is of a kind readily destroyed by incipient putrefaction, the action of proteolytic ferments, or hydrolytic agencies in general. It is of considerable importance, therefore, to be able to control the activity of extracts intended for therapeutic use. The therapeutic applications of the extract are almost all based on the activities demonstrated by physiological experiment. Thus it is used for its properties of raising blood-pressure by arterial constriction (Oliver and Schäfer<sup>1</sup>), accelerating diuresis (Magnus and Schäfer,<sup>2</sup> Schäfer and Herring<sup>3</sup>), exciting contraction of the uterus (Dale,<sup>4</sup> Bell and Hick,<sup>5</sup> v. Fränkl Hochwart and Fröhlich<sup>6</sup>). Blair Bell, who gave the extract its first thorough clinical trial, observed in addition its power of restoring peristal-

<sup>1</sup> Oliver and Schäfer: *Journ. of Phys.*, xviii, p. 277, 1895.

<sup>2</sup> Magnus and Schäfer: *Ibid.*, xxvii, *Proc. Phys. Soc.*, p. ix, 1901.

<sup>3</sup> Schäfer and Herring: *Phil. Trans.*, 1906 B.

<sup>4</sup> Dale: *Biochem. Journ.*, iv, p. 427, 1909.

<sup>5</sup> Bell and Hick: *Brit. Med. Journ.*, i, p. 777, 1909.

<sup>6</sup> v. Fränkl-Hochwart and Fröhlich: *Arch. f. exp. Pathol. u. Therapie*, lxiii, p. 347, 1910.

sis to the paralytically distended bowel, which is not represented by any definite action on the bowel of the normal animal. The galactagogue action (Ott and Scott,<sup>7</sup> Schäfer and Mackenzie<sup>8</sup>) may also prove to be therapeutically valuable.

In choosing, among these different expressions of activity, a suitable basis for standardisation, we are met at the outset by the uncertainty as to whether they are due to one or several principles. Schäfer and Herring pronounced definitely for a diuretic principle distinct from that responsible for the pressor action, but others have found their evidence faulty on some points and inconclusive on others. Again it has recently been stated by Engeland and Kutscher<sup>9</sup> that they succeeded in separating, by a method of fractional precipitation, from an extract of the whole pituitary body, a basic fraction possessing the characteristic action on the uterus, but not that on the blood-pressure. Judging from their records of the effect of the original extract on the blood-pressure they appear to have worked under conditions very little suited to the demonstration of that effect. A dose corresponding to 5 cc. of their original extract caused a small rise of blood-pressure in a rabbit, and a larger fall of pressure in a cat. On the other hand, a not exactly indicated quantity of the basic fraction produced some effect on a cat's isolated uterus, while approximately twice the amount of the same preparation had no perceptible action on the rabbit's blood-pressure and caused a slight fall of pressure in a cat. It does not seem to us that such qualitative evidence, which ignores the much greater sensitiveness of the uterine than the blood-pressure response, seriously affects the question whether the specific pituitary principles, causing rise of blood-pressure and uterine contraction, are identical or distinct. The theoretical possibility that each one of the different actions is due to a separate principle will, however, obviously remain open until a pure principle can be isolated, when it will be possible to discover how much of the activity of the whole extract it possesses. But the

<sup>7</sup> Ott and Scott: *Proc. Soc. exp. Biol.*, New York, 1910.

<sup>8</sup> Schäfer and Mackenzie: *Proc. Roy. Soc. B.*, lxxxiv, 1911; Mackenzie: *Quart. Journ. exp. Physiol.*, iv, p. 305, 1911.

<sup>9</sup> Engeland and Kutscher: *Zeit. f. Biol.*, lvii p. 527, 1912.

point of practical importance for purposes of standardisation is that in the ordinary extract the different types of activity seem to run strictly parallel. That is to say, we have never found an extract which was inferior in pressor action and had not a like inferiority as a diuretic and a uterine stimulant; nor have we succeeded in obtaining evidence of any treatment of the extract, deliberate or accidental, which weakens or destroys one action without weakening all in like degree. It seems safe, then, to adopt, as a criterion of the general activity of the extract, that one of its actions which can be measured with the greatest accuracy, whatever be the ultimate conclusion as to the number of principles involved.

At first sight the action on the blood-pressure, which has furnished by far the most reliable method of standardising suprarenal preparations, would seem the obvious choice. It has, however, very serious disadvantages in the case of pituitary extract. In the first place, the effect is apparently not a pure one, as Schäfer and Vincent<sup>10</sup> indicated.

It may be doubted whether the depressor action, seen with injections subsequent to the first, is wholly due to a different principle. Paton and Watson<sup>11</sup> have recently shown that in the duck a pure fall of blood-pressure is the characteristic effect of the extract. In any case, however, the waning of the pressor action (Howell<sup>12</sup>) and the increasing prominence of the preliminary depression, with injections subsequent to the first, constitute a very serious drawback to the use of blood-pressure effects as a method of comparing the action of two extracts on the same animal. On the other hand, the degree of pressor effect produced, in different individuals of the same species, by equal injections of the same extract, varies so widely, that no method of evaluation can safely be based on it. The difficulty of the declining effect of successive doses in the same animal may to a certain extent be overcome. If a cat with the cord cut in the neck and the brain destroyed be used, no injection being made until, under the artificial respira-

<sup>10</sup> Schäfer and Vincent: *Journ. of Phys.*, xxv, p. 87, 1899.

<sup>11</sup> Paton and Watson: *Journ. of Phys.*, xlv, p. 413, 1912.

<sup>12</sup> Howell: *Journ. exp. Med.*, iii, p. 2, 1898.

tion, all anesthetic has been removed, and the blood-pressure has become low and steady, the first dose, indeed, has an enormously greater effect than the second, while the third has yet smaller result. But if the doses given are small (not more than 0.1 cc. of a 10 per cent decoction of fresh tissue), it will be found that, after about the fourth dose, the effects of subsequent injections though small, are almost uniform. By interposing an injection of the preparation to be tested, at this stage, between two of an extract used as a standard of reference, a marked difference of activity can usually be detected. As indicating the scale of accuracy obtainable, we may say that we could usually rely on seeing an obvious difference between the effects of 0.2 cc. and 0.15 cc. of the same preparation by this method. A closer accuracy might be attained in some experiments; but the method was uncertain, and we frequently had to repeat a standardisation on two or three cats before coming to a definite conclusion.

The diuretic action seems likewise unsuited to the comparison of different preparations. The tolerance produced by a first injection is, indeed, less marked than in the case of the blood-pressure response, as Schäfer and Herring pointed out; but with large doses it is still well-marked, and if small doses are used it may be difficult to distinguish genuine effects from the spontaneous variations of urinary flow which occur in almost any experiment, however constant the controllable conditions. We have not made any experiments on the galactagogue effect, and have no evidence for or against its constancy in relation to the other activities of the extract. It does not, however, seem a likely basis for quantitative comparison, apart from the trouble of securing a regular supply of cats in lactation.

In seeking for a method which would overcome or minimise the difficulty caused by tolerance, we were guided partly by consideration of the probable cause of the phenomenon. The fact that tolerance is absent or, at least, for practical purposes negligible, in the case of the pressor effect of adrenine, has been generally attributed to the ease with which that base is destroyed by oxidation. In the case of a less easily oxidisable substance, such as the pituitary active principle, the restoration of the tissue to its origi-



nal condition of responsiveness may be expected to depend largely on gradual elimination by the kidneys, which is known to take place. The substance is not very readily diffusible, so that the concentration in the circulating fluids is probably reduced but slowly. How far the disappearance of the principle depends on such excretion, and to what extent it is destroyed by tissue ferments, is not known. But it is clear that injection into the intact circulation does not afford theoretically ideal conditions for the rapid return of the tissue to its original condition. It seemed probable that a thin strip of isolated muscle, suspended in a large volume of Ringer's solution, which could be rapidly removed and replaced by a fresh volume, would have a much better chance of recovering its original condition by the rapid washing out of the principle, or the reduction of its concentration below the threshold of activity. We have tried various forms of plain muscle for the purpose, but the only one giving satisfactory results is that of the uterus, which one of us (Dale<sup>13</sup>) showed previously to be exquisitely sensitive to the action of the extract, whether in the body or treated as an isolated organ. Kehrer, who first described the use of the uterus as an isolated organ,<sup>14</sup> has also observed its response to pituitary extract, and Engeland and Kutscher<sup>15</sup> employed the same method in the investigation already mentioned. The isolated horn of the uterus of the non-pregnant cat gives fairly good results, responding with but little diminished contraction to a second dose after a first has been carefully washed away. It is subject, however, to inconvenient spontaneous slow variations of its average tonus, and is apt to acquire a disconcerting rhythm. The uterine horn of the young virgin guinea-pig is greatly superior in these respects, its natural tendency, when left undisturbed in the Ringer's solution, being to acquire a condition of complete relaxation, broken only by a small rhythm. It is very sensitive to the extract, which can, therefore, be given in very small doses, and normally relaxes with promptitude to its original level of minimal tonus on washing out and changing the solution.

<sup>13</sup> Dale: *Biochem. Journ.*, iv, p. 427, 1909.

<sup>14</sup> Kehrer: *Arch. f. Gynäkol.*, lxxxi, p. 160, 1906.

<sup>15</sup> Engeland and Kutscher: *Zeit. f. Biol.*, lvii, p. 527, 1912.

We have used it now for over a year for standardising pituitary extracts, with results which, though not ideal, seem to us an advance on those obtainable by other methods.

## II. DESCRIPTION OF THE METHOD

The following is a description of the arrangement which we have had in use now for some years and have found convenient in this and similar experiments, involving the use of an organ isolated in Ringer's solution, the latter being renewable when necessary without disturbing the organ or the record.

The Ringer's solution in which the organ is suspended is contained in a vessel formed of a wide lamp glass (Fig. 1, *A*) such as is used with an incandescent gas-mantle. The lower end of this is plugged with a rubber bung ( $C_1$ ) having one central bore. Through the latter passes one limb of a wide glass T-tube ( $T_1$ ) which ends flush with the upper surface of the bung, so that the fluid in the lamp-glass can be run out through it to the last drop. This tube passes through a second rubber cork ( $C_2$ ) which fills an opening in the bottom of an outer cylindrical copper vessel (*B*) which forms a constant-temperature water-jacket. The temperature is kept uniform by a device due to Locke, a copper rod (*R*) passing through the water-bath, the openings where it passes the walls being made tight with solder. On this rod hangs a Bunsen burner with a brass chimney, and, by shifting this burner along the rod when necessary, the temperature can easily be kept between 38° and 39°C. The other limbs of the T-piece ( $T_1$ ) are connected by rubber junctions armed with spring-clips (*X*, *X*) to a waste-pipe, through which the lamp-glass is emptied, and a syphon tube (*S*) leading from a large flask which holds warm Ringer's solution for refilling. At the lower end of the syphon another T-piece ( $T_2$ ) is interposed, which serves as a by-pass, allowing the syphon to be filled with warmed Ringer immediately before a change is required. Into the lamp-glass chamber dips a narrow glass tube (*O*). This is turned at a right angle about half an inch from its lower end. The end is sealed into a blob; into this a platino-iridium pin ( $P^{16}$ )

<sup>16</sup> A broken platino-iridium syringe-needle serves admirably.

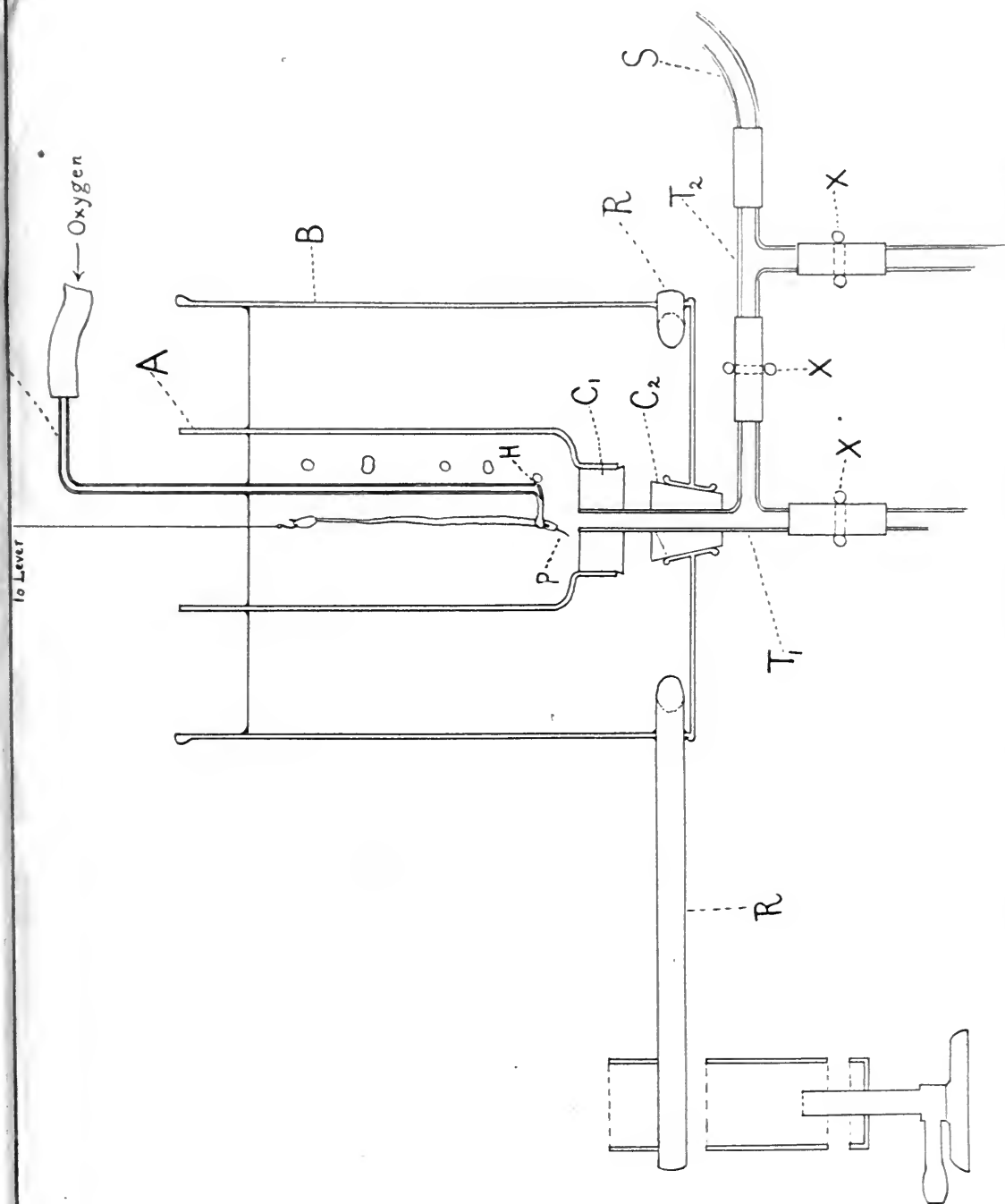


FIG. 1. DIAGRAMMATIC SECTION OF CONSTANT TEMPERATURE BATH FOR ISOLATED ORGANS (SEE TEXT)

is fused. This serves for attaching the lower end of the isolated organ. The upper end of the tube is connected to a bomb of oxygen, and the gas issues through a small hole (*H*) blown at the bend near the lower end of the tube. A continual stream of small oxygen bubbles thus rises through the Ringer's solution, which it oxygenates and stirs at the same time. The hole is so situated that the bubbles do not play directly on the suspended organ. Through the upper end of the latter is passed a small hook, which we usually make from a gilt entomological pin. This is attached by a silk thread to the shorter arm of a lever. The lever is made of a straw cemented to the side of an ebonite pulley, the axle of which furnishes the fulcrum. We generally use an ink writing point, a long paper being used and a flat surface secured by use of two small drums at the recording end of the kymograph (Fig. 2). This is essential for tracing with uniform pressure the long, sweeping curves drawn by contraction of the guinea-pig's uterus, even if a non-magnifying lever is used. A very light load is used and any variation of friction on the drum may disturb the result seriously. Over the pulley to which the lever is attached is fixed a little rubber-faced brake, controlled by a small Bowden cable, as supplied for actuating the release-mechanism of photographic shutters. This serves to fix the lever when required, so that the fluid can be changed without seriously interrupting the record. The ratio between lever-arms which we have usually employed magnifies the contraction about twice.

The temperature of the fluid in the reservoir flask is adjusted by periodical use of a burner beneath and by running in cold Ringer when needed. Some trouble might be saved by having a large reservoir of Ringer's solution in a thermostat, but this is undesirable for another reason. The solution which we use gradually loses  $\text{CO}_2$  when kept at  $38^\circ$  to  $40^\circ\text{C}$ . for any length of time and deposits some of its calcium as carbonate. We shall see that this weakening of the calcium content is specially undesirable for our present purpose. It is better, therefore, to warm a moderate quantity at a time, keeping the main bulk cold. If the Ringer in the reservoir is kept at  $40^\circ\text{C}$ ., and the cooled solution in the syphon replaced by use of the by-pass, the fluid reaches the experimental

bath at a temperature of  $38^{\circ}$  to  $39^{\circ}\text{C}$ . The solution we have used throughout is made up on Locke's formula.<sup>17</sup> The water used was distilled with a glass condenser. Having at one period a series of unsatisfactory results, the sensitiveness of the uterus in several

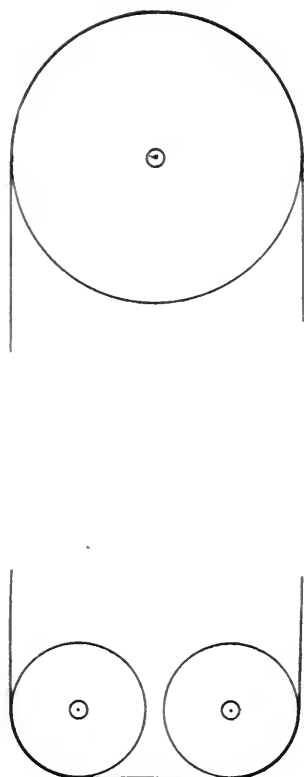


FIG. 2. KYMOGRAPH WITH FLAT WRITING SURFACE

experiments showing a rapid decline with successive doses, we investigated the composition of the Locke-Ringer solution, and discovered that, through an error in calculation, the batch in

<sup>17</sup> NaCl.....	9.0	} in 1 litre of glass-distilled water.
KCl.....	0.42	
CaCl <sub>2</sub> .....	0.24	
Dextrose.....	1.0	
NaHCO <sub>3</sub> .....	0.5	

use had been made up with only half the correct proportion of calcium chloride. On making up a new batch correctly we again obtained the almost uniform response to successive equal doses which is the chief advantage of the method. It is clear, then, that an adequate calcium content of the bathing fluid is an important factor in restoring the sensitiveness of the uterus after an effective dose of the extract. The volume we have used in the bath is 250 cc., with which the lamp-glass chamber which we use is about three-quarters filled. The water in the outside jacket is adjusted to stand at the same level, and serves as a guide to the refilling of the bath with the same volume of fluid at each change.

Young virgin guinea-pigs, of not more than 350 grams weight, usually give the best results. With older animals the uterus is apt to show too much automatic rhythm and to be too sensitive, though it may occasionally give an easily interpreted record. The animal is killed by a blow on the head and bled out as completely as possible. Some experiments undertaken for another purpose suggest that more uniformly good results might be obtained by perfusing the uterus thoroughly with the Ringer's solution, through the aorta, before excision; but the procedure is probably not worth the trouble involved for routine use. All the results dealt with in this paper have been obtained with the organ simply excised and suspended after bleeding the animal. A horn of the uterus is removed by cutting through the broad ligament close to it along its entire length. The incision is carried forward so as to excise the ovary as well, which is left attached to the uterus by the fold of broad ligament in which the Fallopian tube runs. The pin at the lower end of the oxygen-tube is pushed through the lower end of the horn, and the hook suspended from the recording lever is passed through the ovary. The oxygen-tube is then lowered into the bath of Ringer's solution, to such a depth that the lever is pulled well beyond the horizontal position. The manipulation and exposure, followed by immersion in the warm bath, will be found almost invariably to produce a high degree of tonus. When the uterus is left to itself the tone slowly gives way, with small rhythmic interruptions, until a condition of almost complete relaxation is attained. The ideal uterus for our

purpose shows small rhythmic contractions rising from a steady level of minimum tonus. If the uterus is rather large and the rhythm excessive the load on the lever may be increased by a small weight. For the very slender horns of young virgin uteri the excess weight of the longer arm of the straw lever, with the brass ink-writing point at its tip, has usually been found a sufficient load and has needed partial counterpoising in some cases.

There seems to be some difference in the automatic activity of the uterus at different times of the year, and we have found it, on the whole, more quiescent, and more regularly responsive to the pituitary extract during the winter months. At the same time quite good results are obtainable during the summer months; all the tracings reproduced in illustration here were obtained during this July.

When the uterus has attained the condition of uniform low tonus, which usually takes from fifteen to thirty minutes after suspension in the bath, the first dose of pituitary extract is added. The dose should be chosen so as to produce a nearly, but not quite maximal tonus, the lever rising with a steady sweep, broken only by slight rhythmic pauses or trifling relaxations. If, after rising to a position of partial tonus, the lever falls again and a wide rhythm ensues, the dose is too small, or, in rare cases, the uterus is unsuitable for the test. A few trials may be necessary to determine the appropriate dose. We usually begin with  $1/100$  to  $1/80$  cc. of our standard extract, to which we shall refer again later. If that is not enough the Ringer's solution is changed and the uterus allowed to relax again. After five to ten minutes at minimal tonus a larger dose ( $1/50$  or  $1/40$  cc.) is then tried. If this is not sufficient it is best to discard the uterus and put up another. Figure 3 shows two successive responses of a fairly good uterus to  $1/80$  cc. of the same extract. In rare cases this dose may be too large, i.e., produce repeated supramaximal responses. In that case smaller doses, e.g.,  $1/200$  cc. are used. The dose having been found which gives the desired smooth rise to nearly maximal tonus the Ringer is changed, and after relaxation and the usual interval of normal tracing, the same dose of the preparation to be compared with the standard is added to the

bath. If this is approximately of standard strength the curve traced will be almost indistinguishable from that traced after the standard dose. On the other hand, if the preparation is definitely weaker than the standard, and if the dosage has been chosen



FIG. 3. THIS AND FOLLOWING FIGURES (EXCEPT FIG. 7) REPRESENT RECORDS FROM THE ISOLATED UTERUS OF THE VIRGIN GUINEA-PIG IN 250 CC. OF RINGER'S SOLUTION.

At A, 0.0125 cc. standard (20 per cent) infundibular extract added to the bath; at B, the same; at RR (in this and succeeding figures), change to fresh Ringer.

rightly, so as not to be supramaximal, the summit of the second curve will fall clearly below that of the first. If the second curve rises slightly higher or lower than the first, this is not necessarily



significant, since a second equal dose of the same preparation will frequently give a slightly bigger effect than the first, or may, on the other hand, cause a slightly smaller one. The uniformity of result is more complete with some uteri than others, and in almost all cases tends to increase with doses succeeding the second. It depends to a considerable extent on the uniformity of interval

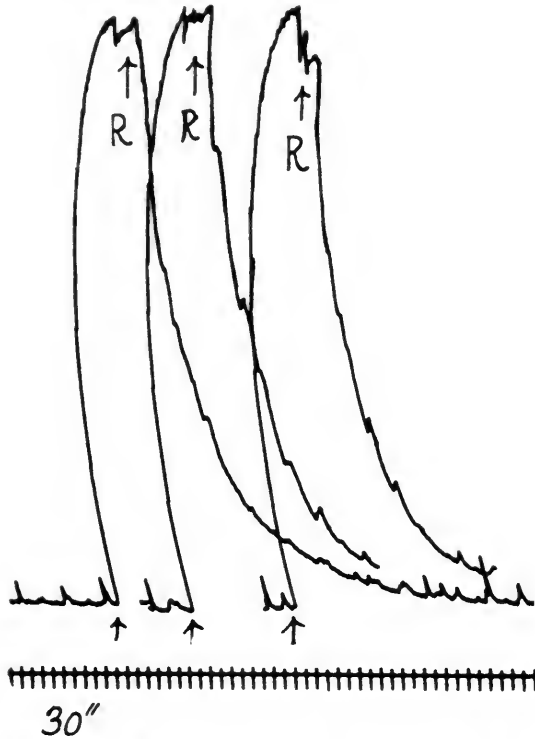


FIG. 4. THREE SUCCESSIVE RESPONSES TO 0.025 CC. OF THE SAME EXTRACT, OVERLAPPED FOR COMPARISON

between the doses, so that the first dose of a group, either at the beginning of the experiment, or at a later stage following a prolonged interruption, tends to produce a slightly abnormal result in one direction or the other. This can be seen in Figures 8 and 10 where the first of a group of doses, given after a prolonged interruption in each of these cases, produces a slightly greater contrac-

tion than the same dose later in the group, the intervals between the doses within the group being approximately ten minutes in each case. Figure 4 shows a group of three contractions produced by successive equal doses of the same preparation. It will be seen that the first dose of this series produces a slightly smaller effect than either the second or third—that is to say, the first dose after an interval in this case causes a temporary increase in the sensitiveness of the uterus. With a long series of doses at short

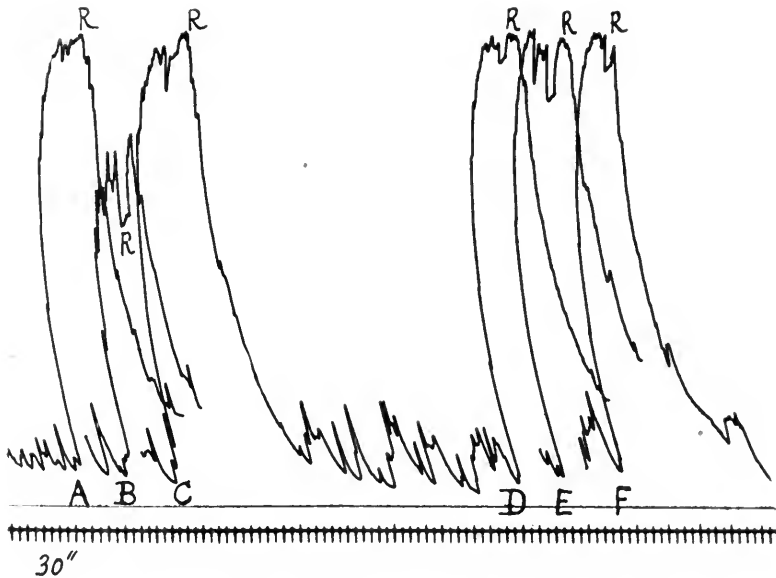


FIG. 5. COMPARISON OF EXTRACT T WITH STANDARD EXTRACT S

At A, 0.0125 cc. S; at B, 0.0125 cc. T; at C, 0.0125 cc. S; at D, 0.02 cc. T; at E, 0.0125 cc. S; at F, 0.02 cc. T.

intervals a gradual decline in response takes place, and it is better, in performing a series of comparisons with the same uterus, to give a group of at most four or five doses at ten minute intervals, then allow an interval of twenty minutes or so and give another group, regarding the first member of each group of curves as probably abnormal. Uteri vary greatly as regards the length of time during which they give regular responses; with a good specimen reliable results can be obtained throughout an ordinary working

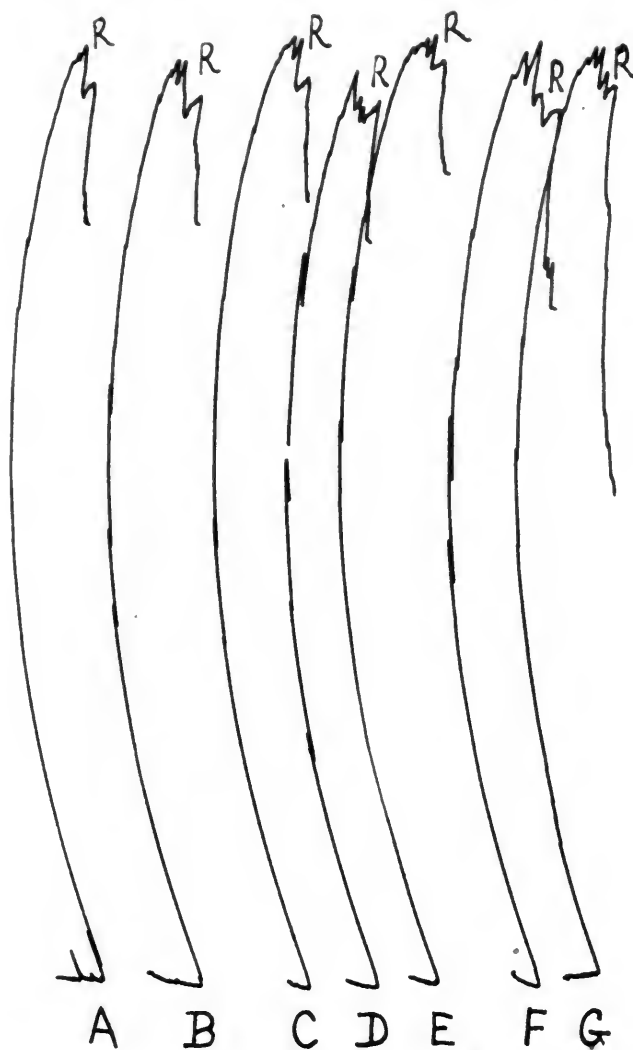


FIG. 6. THE SAME EXTRACTS AS IN FIGURE 5 COMPARED WITH ANOTHER (MORE HIGHLY SENSITIVE) UTERUS

At A, 0.0075 cc. S; at B, 0.0075 cc. T; at C, 0.0075 cc. S; at D, 0.0075 cc. T; at E, 0.0075 cc. S; at F, 0.005 cc. S; at G, 0.0075 cc. T.

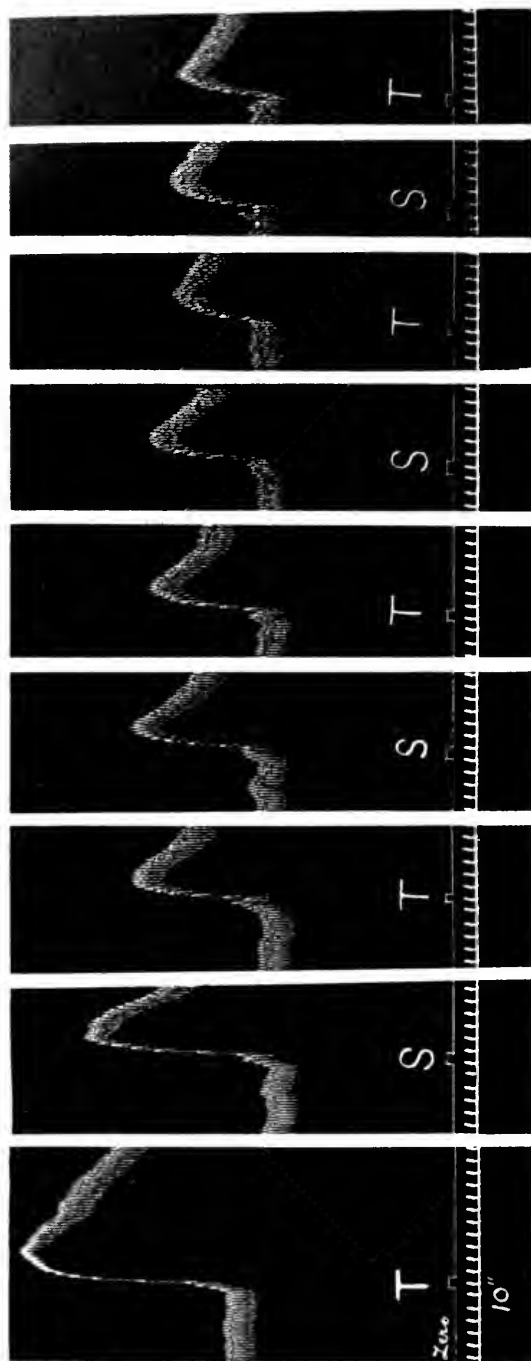


FIG. 7. THE SAME EXTRACTS AS IN FIGURES 5 AND 6 COMPARED ON THE BLOOD-PRESSURE OF A PITHED CAT. DOSE IN EACH CASE 0.05 CC. INJECTED BY THE FEMORAL VEIN

day. We have deliberately emphasized those sources of error which demand care and experience for their complete elimination. At the same time it should be made clear that the precautions mentioned above are only needed for the application of the method with a fineness of discrimination altogether beyond the range of

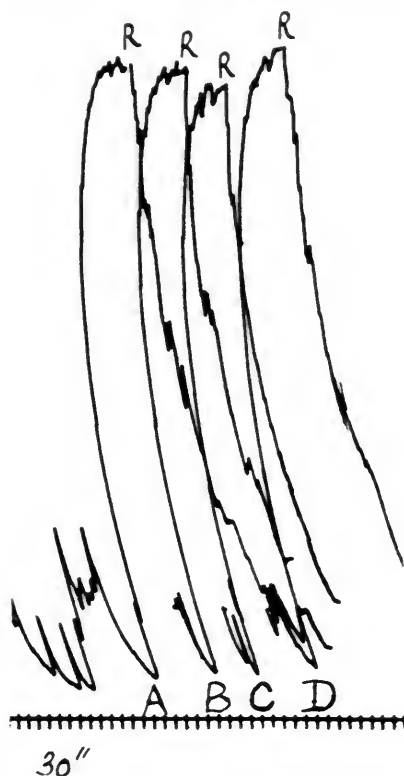


FIG. 8. COMPARISON WITH THE STANDARD (S) OF A NEARLY EQUIVALENT EXTRACT (X)

At A, 0.0125 cc. X (abnormal effect of first dose); at B, 0.0125 cc. S; at C, 0.0125 cc. X; at D, 0.0125 cc. S.

differences appreciable by other methods. In our experience a difference of activity which is only just appreciable by the blood-pressure method under the best conditions is at once obvious by the test on the uterus without any special care in controlling the regularity of the response. Such a case is shown in Fig. 5, which

illustrates the comparison with the standard of a preparation decidedly deficient in activity. It will be seen that the specimen (T) gives in equal doses with the standard (S) produces a very obviously smaller effect. It needs about 0.02 cc. of T to produce the same effect as 0.0125 cc. of S. On another more sensitive uterus (Fig. 6) the equivalent doses were found to be 0.0075 T to



FIG. 9. CONTINUATION OF FIG. 8

At A, 0.0125 cc. S; at B, 0.014 cc. X; at C, 0.0125 cc. S

0.005 S. Certain limitations of the method, with the corresponding precautions desirable, can be gathered from inspection of Figure 6. It will be seen that the uterus which is highly sensitive to minute doses does not necessarily exhibit so great a discrimination between doses of different value as a less sensitive organ, such

as that which gave Figure 5. It will be seen, moreover, that the tendency of the highly sensitive uterus is to give increasing rather than diminishing responses to successive equal doses. By being careful to alternate the doses, however, and to compare each effect with that immediately preceding and succeeding it, the slow increase of sensitiveness is discounted, the inferiority of T made

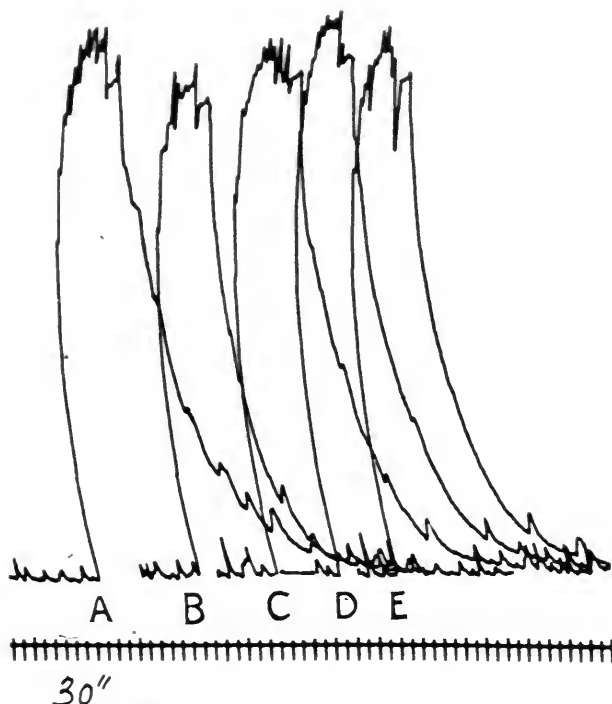


FIG. 10. VARYING DOSES OF THE SAME EXTRACT. UTERUS OF RATHER LOW SENSITIVENESS

At A, 0.025 cc. (abnormal effect of first dose); at B, 0.02 cc; at C, 0.025 cc; at D, 0.03 cc.; at E, 0.025 cc.

abundantly clear, and the approximate equivalence of 0.0075 T to 0.005 S established. This tracing shows the discriminating power of the method at less than its best and we have reproduced it for that reason. It is instructive to compare these results with the attempt to standardise the same preparation (T) against the same standard specimen (S) by the effect on the blood-pressure.

Figure 7 shows the declining pressor effects produced by alternating equal doses of the two. By careful comparison in the later stages it can be made out that the line joining two S summits always falls above the intervening T summit, and the conclusion may be drawn that T is probably inferior in activity. But it would be difficult by this method to get any quantitative idea of the deficiency: in fact it may be regarded as probable that T would be passed as corresponding sufficiently to the standard by any observer who was not aware, from comparative experience with the uterus method, that a deficiency recognisable at all by the blood-pressure test is probably of serious dimensions. Figures 8 and 9 illustrate the recognition of a much smaller difference in activity—practically a 10 per cent difference. Figure 10 illustrates the effect of a group of varying doses of the standard preparation, showing that, discarding the first contraction as abnormal, doses in the ratio 4:5:6 give very clearly differentiated effects. In this case the accuracy is, therefore, 20 to 25 per cent. As a rule we do not trouble to push the estimation to closer limits for standardising a preparation for practical use; but greater accuracy when required, as in renewing the standard, is attainable, as we have shown.

#### CHOICE AND MAINTENANCE OF A STANDARD

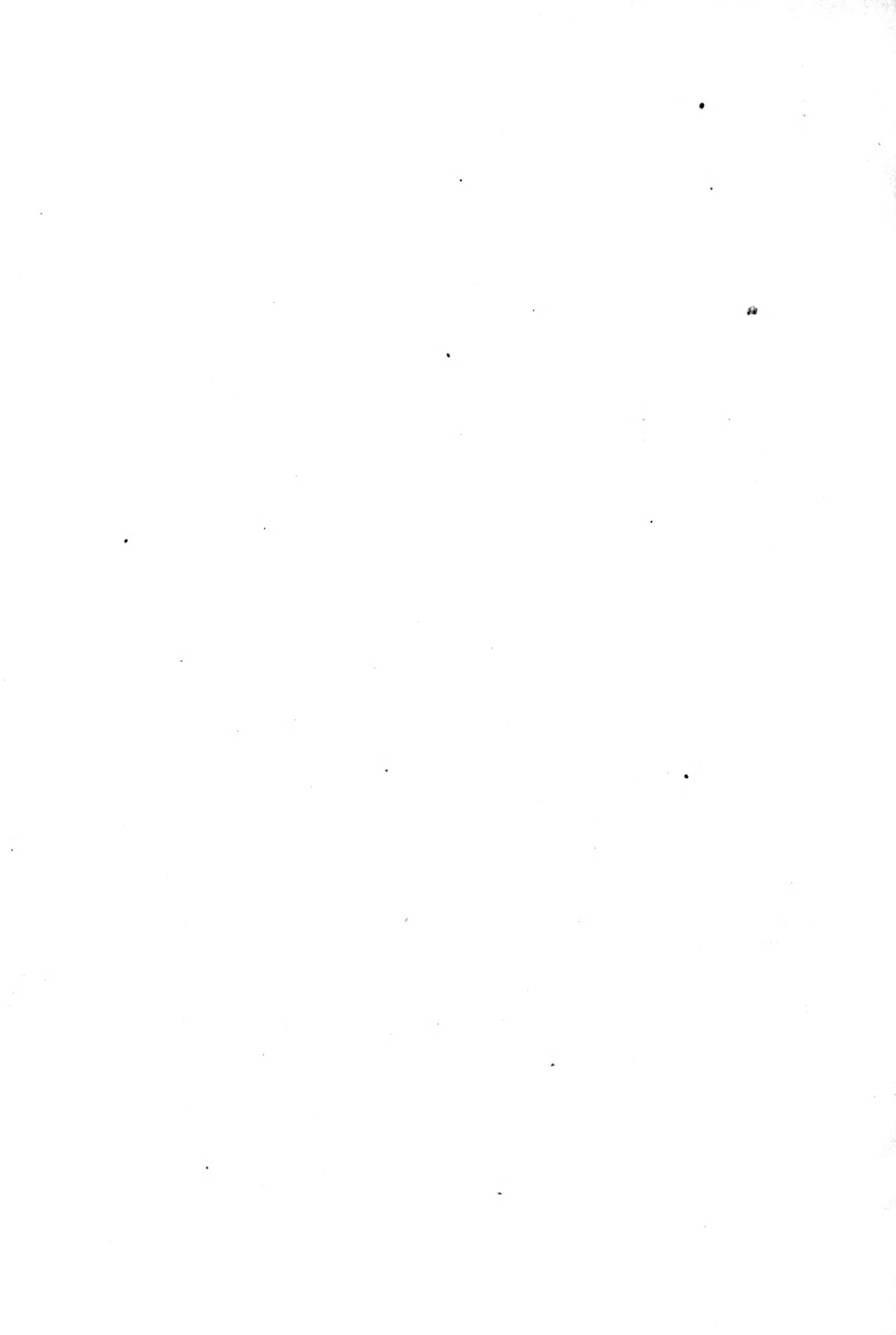
This is a point on which some generally acceptable convention is ultimately to be desired, but further experience of the conditions of stability is needed before any definite recommendation can be given. Schäfer and Herring state that the fresh infundibular substance, dried at low temperature, is indefinitely stable, and it may be that ultimately a freshly prepared decoction of such dried material will prove to be the best standard of reference. But Schäfer and Herring's statement was evidently based on general impression—the effects produced, that is to say, did not become obviously weaker on the average. The question is by no means a simple one, for no absolute standard of reference exists, on the one hand, which can be with certainty reproduced, while, on the other hand, the available methods of evaluation,



including the one we have described, are essentially comparative. The variation in the responsiveness of different individuals, or different isolated uteri, is very wide, so that neither the minimal effective dose nor the magnitude of response to a dose of standard size gives any useful information. For the present we used as a standard the extract prepared by a brief boiling of the perfectly fresh and finely pounded infundibular material with a definite proportion of acidulated water, so as to produce a 10 per cent or 20 per cent extract of the fresh moist substance. The extract is then sterilised by brief autoclaving in small phials. Some activity is lost in autoclaving, but the preparation thereafter has great stability. The use of small phials, of which one can be used for each test, obviates repeated sterilisation, which is inadmissible. At frequent intervals a new batch is chosen which has an activity equal to or just perceptibly greater than that of the former standard, so that the possibility of a very slow lowering of the standard is eliminated, though we have as yet no evidence of deterioration under such conditions.

#### SUMMARY

A method of comparative physiological estimation of the activity of pituitary (infundibular) extracts is described, the test organ being the isolated uterus of the virgin guinea-pig, which, when certain precautions are observed, is found to give a very uniform series of responses to successive equivalent doses. This method is found to detect differences of activity which escape recognition by the blood-pressure test, but appreciable differences of pressor activity always correspond to large differences of action on the uterus in the same direction. The action on the uterus may be adopted, therefore, as a criterion of the general activity of the extract. The method is essentially comparative and not absolute, so that an arbitrary standard must be adopted and renewed at intervals.



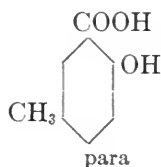
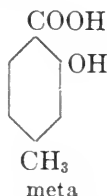
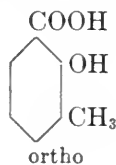
# THE THERAPEUTICAL ACTION OF THE CRESOTINIC ACIDS

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The attention of pharmacologists has been from time to time directed to the cresotinic acids ( $C_6H_3.OH.COOH.CH_3$ ) on account of their close chemical relationship to salicylic acid ( $C_6H_4.OH.COOH$ ) and although this has resulted in the publication of several researches, yet hitherto no exact estimate has been made of their clinical efficacy or relative therapeutical value. There are ten isomeric cresotinic acids, three of which correspond to the three cresols ( $o-m-p-C_6H_4.OH.CH_3$ ) and are known as ortho-, meta-, and para-cresotinic acids respectively.

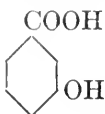



It was with the sodium salts of these that the observations here recorded were made. In all three the OH group stands to the COOH group in the same position as it does in salicylic

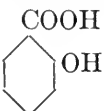
acid  , while the  $CH_3$  group occupies in reference to the

OH group the ortho, meta, and para, positions respectively. As we shall see, the position of the methyl group modifies the action

somewhat, but only to a very limited extent, and quantitatively rather than qualitatively. It is well known that m-oxybenzoic

acid  and p-oxybenzoic acid  do not possess

the strong antiseptic and antirheumatic powers of o-oxybenzoic

(salicylic) acid , and it has been generally assumed that

this difference in action is due to the slight differences in chemical constitution. This may or may not be the reason, but these three cresotinic acids resemble salicylic acid closely in their actions and exactly in the relative positions of their carboxyl and hydroxyl groups. Whether the action depends on the relative positions of these two groups I am unable to say. Theoretically it is a point which should easily be capable of proof, as, out of the remaining seven cresotinic acids, six have a different relationship of the COOH and OH, but I found that I was unable to make or procure any of these in a state sufficiently pure for making observations to decide the question.

As regards previous researches, Buss in 1876 found that "sodium cresotinate" acted in feverish conditions generally and in rheumatic fever especially, very much like salicylic acid. The substance he used was almost certainly a mixture of the above three acids. Using a mixture of the acids (as Buss had done), Koranyi,<sup>1</sup> giving 5 to 6 grams daily in three doses, reports very highly of its value in typhoid fever as an antipyretic, while Gatti<sup>1</sup> found that it reduced the temperature in typhoid, phthisis, and pneumonia.

In 1888 Demme made a careful investigation of the therapeutic action of sodium para-cresotinate, and decided that it was less efficient than sodium salicylate, but at the same time less apt to cause unpleasant side-effects. He states that the meta-

<sup>1</sup> Quoted by Henne.

cresotinate is "therapeutically less active," but he gives no particulars and no cases, and in this conclusion I think he was mistaken. In 1890 Henne published a thesis in which he gives an account of the pharmacological action of sodium para-cresotinate on animals. In 1909 May working with the chemically pure sodium salts of the three acids (p-m-o) found that in rabbits the ortho- and meta-cresotates of sodium had very much the same lethal dose as sodium salicylate and that the para-cresotinate was less poisonous. In checking yeast fermentation he found the meta- and ortho-acids slightly more active than salicylic acid, while para-cresotinic acid was decidedly weaker. Somewhat similar results were got as regards their action on a streptococcus and bacillus coli. All four acids in weak solution inhibited the action of emulsin, ptyalin, and pepsin. May also made two observations on the treatment of acute rheumatism with sodium ortho-cresotinate, finding that it acted satisfactorily and caused no discomfort except profuse perspiration.

For the present research I obtained the sodium salts of o-m- and p-cresotinic acid from Merck and purified them by recrystallizing from spirit. All give a deep purple color with ferric chloride. When 20 grains was dissolved in half an ounce of water the sodium ortho-cresotinate gave a purely sweet taste like sodium salicylate, the meta-cresotinate was less sweet and slightly bitter, the para-cresotinate was bitter and rather harsh with no trace of sweetness.

#### SODIUM ORTHO-CRESOTINATE

Nine cases of acute rheumatism of ordinary severity were treated. The remedy was given usually in 15 to 20 grain doses every four hours, and from 330 to 600 grains were given altogether in each case. It was generally stopped as soon as all objective and subjective symptoms had subsided. A reference to the accompanying charts of four of these cases will show that the course of events is the same as with salicylates. The pain is lessened almost at once, temperature falls rapidly and with it the pulse and respiration, while some time longer is required for the effusion into the joints to disappear.

In cases 1 and 2 the result was in every way satisfactory, but charts 3 and 4 show very definitely the slowing and depressing action on the heart which was noticed in animals both by Demme and May. In both these cases the pulse-rate fell greatly and there was also marked flushing of the face and skin. This action on the heart and circulation is a very decided objection to the use of the ortho-cresotinate, as it prevents it being pushed to large doses, for, even with moderate doses, as the charts indicate, great caution is necessary. Its action as a specific in acute rheumatism is fully as powerful as that of sodium salicylate, and, in such doses as I gave, deafness or ringing in the ears was never observed, while the sweating was much the same as with salicylate.

#### SODIUM META-CRESOTINATE

Thirteen cases of acute rheumatism and one of erythema nodosum were treated with the meta-cresotinate, mostly in 15 or 20 grain doses every four hours (90 to 120 grains daily), but in one case 240 grains were given during the first day (20 grains two hourly), and in another 160 grains daily for three days. In most of the cases the administration of less than one ounce of the salt sufficed to get rid of the pain, fever, and swelling of the joints, but it was also continued till about 800 grains, and in two cases, till 1440 and 2040 grains were reached. In none of these were any poisonous or unpleasant effects noticed except moderate perspiration. The accompanying charts of some of the cases treated show that its efficacy is very much the same as that of sodium salicylate. As seen in chart 5, treatment was begun on the first day of illness and was very satisfactory, while in figure 6 an equally satisfactory result is shown. In chart 7 the dose required to be increased and treatment was more prolonged, probably owing to the presence of pericarditis, and the same thing is seen in chart 8. The course of the erythema nodosum case is shown in chart 9. Mild cases of erythema nodosum often run much the same course as this without any treatment, and it is difficult to estimate the influence of the drug on it. On the whole, sodium meta-cresotinate acts well in acute rheumatism.

It is difficult to judge accurately how it compares with sodium salicylate as rheumatic cases differ so much in their reaction to salicylic treatment, but it is probably not quite so powerful, although the difference between the two must be small.

#### SODIUM PARA-CRESOTINATE

Two cases only of acute rheumatism were treated with the para-cresotinate. As Demme pointed out, its action is distinctly less powerful than salicylate, and as I also found this to be the case I did not multiply my observations. In chart 10 the temperature fell slowly and only after an increase of dose, while in chart 11 the symptoms did not subside until the metacresotinate had been substituted for it. No unpleasant effects were observed in either case.

I also made some observations with methyl meta-cresotinate. It is a yellowish liquid with a powerful penetrating odor very like methyl salicylate, over which it possesses no advantages for clinical use.

The action of the meta- and ortho-cresotates of sodium was also tested in a number of cases of chorea, of gonorrhoeal rheumatism, of chronic muscular rheumatism (fibrositis), and of scarlet fever, as well as in septic arthritis, rheumatoid arthritis, influenza, and hay fever, but like sodium salicylate they proved to be entirely without any specific curative effect in these conditions.

#### SUMMARY

1. The sodium salts of o-m- and p-cresotinic acids have a powerful specific action in acute rheumatic conditions.

2. They are for practical purposes inferior to sodium salicylate: (1) The ortho-cresotinate, even in comparatively moderate doses, slows and depresses the heart; (2) the para-cresotinate is not nearly so efficacious; (3) the meta-cresotinate, while very similar in strength, has no advantages over the salicylate.

3. So far as I tested them, they have no therapeutical actions which are not possessed by sodium salicylate.

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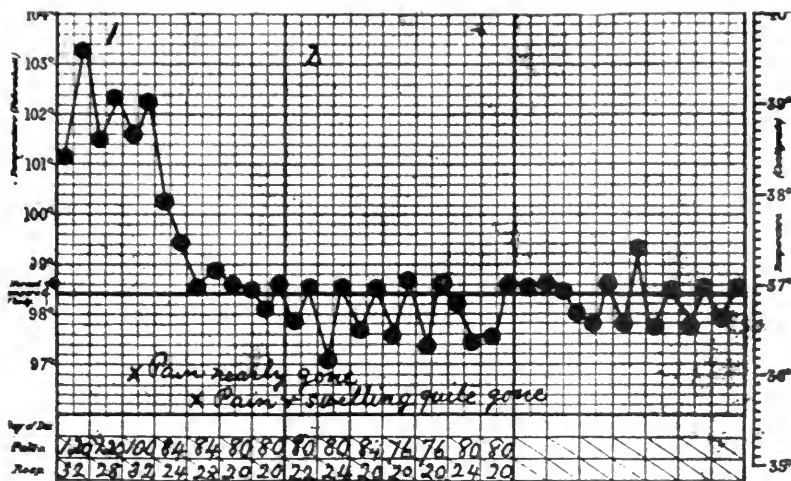


CHART 1. MAN, 32. ACUTE RHEUMATISM

Both knees, both ankles, and right wrist swollen and painful. Four days ill. Previous attack six years ago, chorea at twelve years. 1, Sodium orthocresotinate, 15 grains 4 hourly; 2, stopped; 540 grains given.

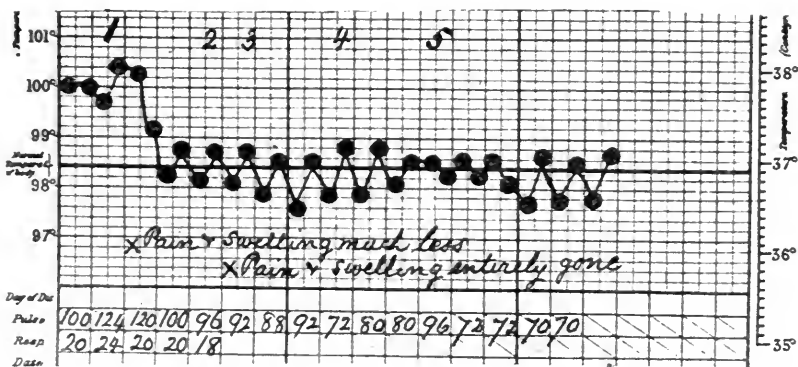


CHART 2. MAN, 23. ACUTE RHEUMATISM

Many joints affected. Ill one month at sea, no treatment. 1, Sodium orthocresotinate, 15 grains 2 hourly; 2, 15 grains 4 hourly; 3, 10 grains 4 hourly; 4, 10 grains 3 daily; 5, stopped; 480 grains given.



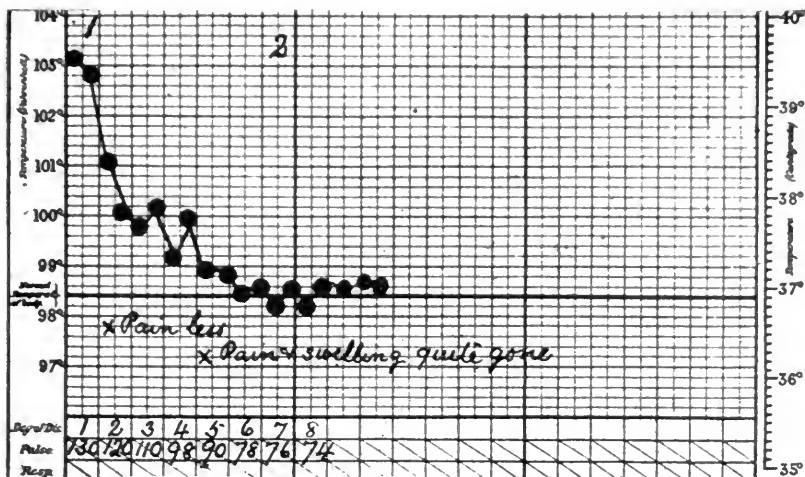


CHART 5. MAN, 24. ACUTE RHEUMATISM

Both ankles swollen and painful, knee joints painful. 1, sodium metacresotinate, 15 grains 4 hourly; 2, stopped; 540 grains given.

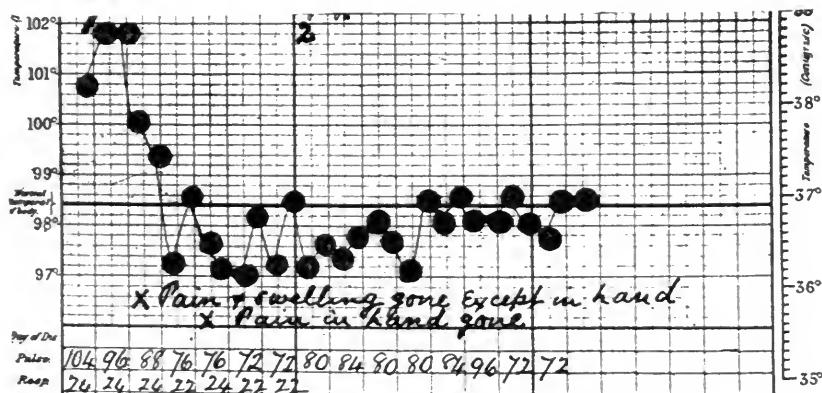


CHART 6. MAN, 19. ACUTE RHEUMATISM

First attack 7 years ago, followed by chorea. A second attack two years ago. Mitral systolic murmur. Both knees and both ankles swollen, red and painful. The right hand and an area in lumbar region much swollen and tender. 1, SODIUM METACRESOTINATE, 15 grains 4 hourly; 2, stopped; 630 grains taken in all.

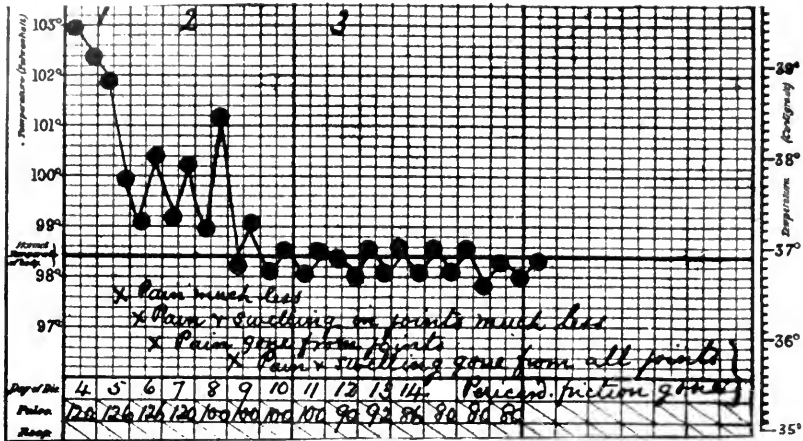


CHART 7. MAN, 20. ACUTE RHEUMATISM

Knees, ankles and wrists swollen and painful, hip-joints painful. Second attack, mitral murmur, aortic murmur, pericarditis. 1, Sodium metacresotinate, 15 grains 4 hourly; 2, 20 grains 6 hourly; 3, stopped; 780 grains given.

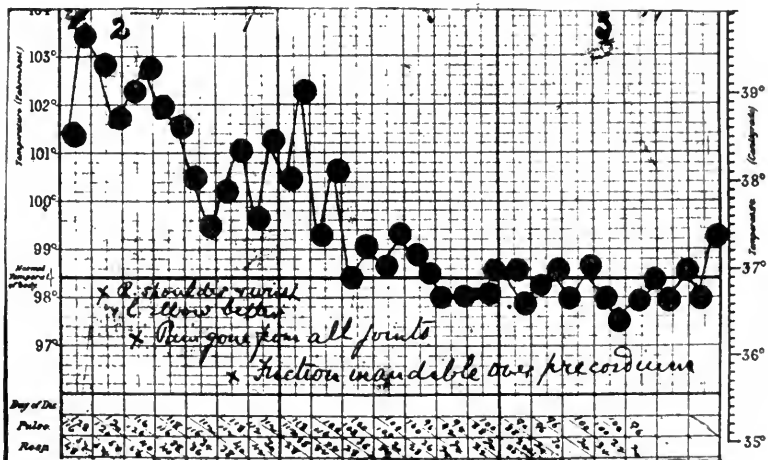


CHART 8, MAN, 30. ACUTE RHEUMATISM

Pain and swelling in right shoulder and wrist, and left elbow and knee, and both ankles. Marked pericarditis. 1, Sodium Metacresotinate, 20 grains 2 hourly; 2, 20 grains 4 hourly; 3, stopped; 2040 grains taken.

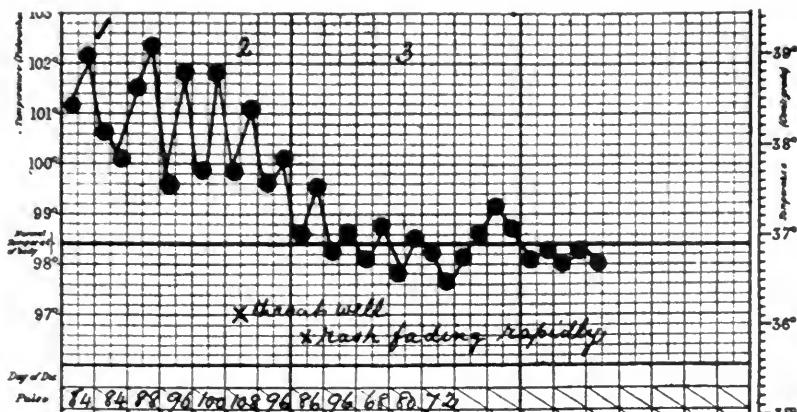


CHART 9. WOMAN, 27. ERYTHEMA NODOSUM

Severe eruption on legs and arms, tonsillitis, pains in joints. 1, Sodium metacresotinate 20 grains 4 hourly; 2, tonsillitis well; 3, stopped.

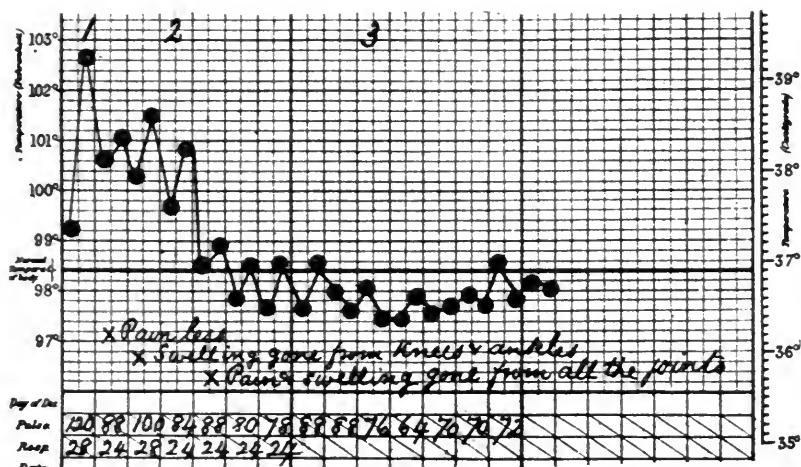


CHART 10. MAN, 21. ACUTE RHEUMATISM

Ankles, knees, toes, wrists and elbows are swollen and painful. Four days ill. No complications. 1, Sodium paracresotinate, 15 grains 4 hourly; 2, 15 grains 3 daily.

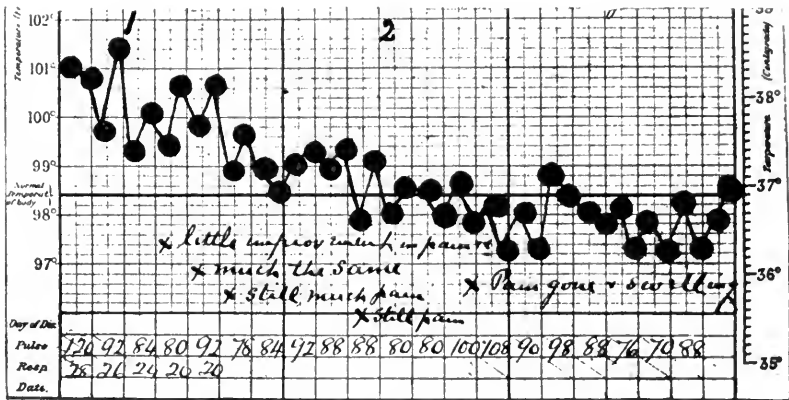


CHART 11. MAN, 23. ACUTE RHEUMATISM

Pain, redness and swelling in knees, wrists, and great toes. 1, SODIUM PARACRESOTINATE 20 grains 6 hourly; 540 grains in all taken; 2, SODIUM METACRESOTINATE 20 grains 4 hourly; which was followed by much more rapid improvement.

## THE ACTION OF HYDROXY-CODEINE

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Hydroxy-codeine is an alkaloid recently discovered in opium by T. and H. Smith of Edinburgh, the well known firm of manufacturing chemists. It was handed over by them to Drs. J. J. Dobbie and Alexander Lauder for chemical examination and they have published in the Proceedings of the Chemical Society<sup>1</sup> a short account of it. Its percentage formula is  $C_{18}H_{21}NO_4$ , which differs from that of codeine merely in possessing an additional atom of oxygen. It is a tertiary base combining with one molecule of methyl iodide to form a crystalline methiodide and although the alkaloid itself has not been obtained crystalline, its hydrobromide ( $C_{18}H_{21}NO_4HBr$ ) crystallises readily from water and spirit in large hard pure prismatic crystals, and its platinum salt has the formula  $(C_{18}H_{21}NO_4)_2H_2PtCl_6$ . It appears to be an hydroxy-derivative of codeine. Its absorption spectra agree closely with those of codeine, and its color reactions are identical.

In the following experiments to determine the pharmacological action of the new alkaloid, the hydrobromide was used, a large supply of which was furnished by Messrs. T. and H. Smith for the purpose. It was in absolutely white crystals and was conveniently soluble in cold water. A very few experiments sufficed to determine that its action is very similar to that of codeine, as was to be expected from their close chemical relationship. Its toxicity however is somewhat less. Stockman and Dott<sup>2</sup> found that in frogs very small doses of codeine (0.0013 gram) had no apparent action till the next day, when there was a faint increase in the spinal reflexes, but that doses of 0.005 gram and

<sup>1</sup> Vol. xxvi, 339, 1910.

<sup>2</sup> Proceedings Royal Society of Edinburgh, vol. xxvii.

upwards caused the characteristic stage of depression with diminished reflexes (common to most of the opium alkaloids), followed by a stage of increased reflex excitability. With codeine the narcotic stage is not deep nor prolonged, and the duration and severity of the stage of increased reflex excitability depends on the amount given. After 0.01–0.03 gram tetanus always develops, and sometimes so quickly that it is often difficult to observe any narcotic stage. 0.02 gram is usually a fatal dose for a medium sized frog (*R. temporaria*). Comparing these results with those obtained from hydroxy-codeine, I found that the latter was practically identical in action, but that somewhat larger doses were required, as the following experiment shows. At 11.47 0.005 gram was given hypodermically to a medium sized frog in 0.5 cc. water. At 1.30 there was little effect, but the animal was slightly drowsy. At 5.00 the spinal reflexes were distinctly increased, and this lasted for four days, the animal gradually returning to normal. Smaller doses (0.002 gram) did not cause lethargy, but several hours later there was a slight increase in the reflex excitability. Large amounts, such as 0.02 to 0.05 gram bring on tetanus almost at once, or in thirty or forty minutes, along with a very varying degree of narcotic depression and some paresis of the motor nerves. Once tetanus is fully established, the convulsions lead to exhaustion and death in a comparatively short time. Direct injection of the alkaloid into the aorta causes tetanus without any preceding stage of spinal depression. Division of the spinal cord at the medulla does not alter the symptoms.

In rabbits codeine can never cause more than very slight narcosis, for, when the dose is increased, this, instead of deepening the narcosis, brings about increased reflex excitability. The minimum lethal dose for medium sized rabbits is 0.06 to 0.1 gram, and when such amounts are given the narcotic state is ill-defined or absent and tetanus supervenes almost at once. Slight drowsiness comes on after 0.01 to 0.03 gram hypodermically. With hydroxy-codeine, 0.02 to 0.04 gram per kilo of body weight causes slight depression, the animal sits quietly for an hour or thereabouts and is a little drowsy, but there is no change in the



pupil or in the rate of respiration and heart-beat. With 0.08 gram per kilo the narcotic stage is very apparent, the rabbit lies on its belly with its legs spread out, but at the same time it is easily roused, is nervous and tremulous, and very soon the reflexes show an increase, and tetanic spasms may come on in fifteen to forty-five minutes after administration. It gradually recovers from this dose. A larger dose (0.13 gram per kilo) is fatal, with indefinite narcosis and very severe spinal convulsions.

With the smaller doses no definite action was observed on the respiration, pulse, or blood-pressure, but with the larger doses the respiration was somewhat slowed, the blood-pressure remaining practically unaffected.

Hydroxy-codeine was also used therapeutically in the same class of cases in which codeine has been found of value. The amount given was generally  $\frac{1}{4}$  to  $\frac{1}{2}$  grain repeated several times during the day. In controlling colicky spasm, in pulmonary cough, in irritation of the throat, and in similar conditions, it was found inferior to codeine and very much inferior to small doses of morphine. The patients rapidly became accustomed to it, and it seemed to lose its action. It did not control diarrhoea, and in one case of diabetes it failed to diminish the output of sugar, but probably the doses given were too small.

#### SUMMARY

1. Hydroxy-codeine has practically the same action as codeine, but is somewhat less active.
2. As a therapeutical agent it is inferior in efficacy to codeine.



## THE EMETIC ACTION OF THE DIGITALIS BODIES

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### DISCUSSION OF THE LITERATURE

Emesis follows the use of large doses of digitalis so commonly that it could not fail to attract attention with the introduction of the drug into therapeutics, and Withering<sup>1</sup> quotes an earlier authority as follows: "It is a medicine which is proper only for strong constitutions, as it purges very violently, and excites excessive vomitings. Ray. Hist. 767."

Withering observed the nature of the emetic action of digitalis to which he calls attention in the following words: "It is curious to observe that the sickness, with a certain dose of this medicine, does not take place for many hours after its exhibition has been discontinued;" and he adds, "The sickness then excited is extremely different from that excited by any other medicine; it is peculiarly distressing to the patient; it ceases, it recurs again as violent as before; and then it will continue to recur three or four days, at distant and more distant intervals."

The consensus of opinions among clinicians and pharmacologists is that the various members of the digitalis group induce gastrointestinal disturbances through the irritant action which they exert directly upon the mucous membranes of the stomach and intestines. This view is held almost universally with regard to digitalis and digitoxin, and somewhat less generally concerning strophanthus and amorphous strophanthin.<sup>2</sup>

<sup>1</sup> The Foxglove, Birmingham, 1785.

<sup>2</sup> Strophanthin, or amorphous strophanthin, as it is sometimes called to distinguish it from ouabain, the so-called crystalline strophanthin, is the active principle of strophanthus, and the actions of strophanthus and strophanthin probably differ only in degree.

The literature relating to the digitalis bodies is very extensive and no attempt will be made to discuss more than a few of the experiments which have been designed to determine the nature of the action which they exert upon mucous membranes.

Gubler<sup>3</sup> applied digitalis and Nativelle's digitaline, which is practically identical with digitoxin, to denuded skin and mucous surfaces and observed that they caused inflammation proceeding to ulceration. In a later publication<sup>4</sup> he states that the stomach is protected from the effects of small doses of digitalis through rapid envelopment in mucus.

Rabuteau<sup>5</sup> came to conclusions opposed to those of Gubler, but his experiments were so crude that they hardly require further consideration.

Koehnhorn,<sup>6</sup> who has given an excellent account of the study of a case of fatal chronic digitalis poisoning in man, observed isolated patches of hyperemia with ecchymoses at the post mortem examination of the stomach of a man who had taken an amount of digitalis estimated at 13.5 grams, in a period of five weeks. He states that the gastric symptoms which had been observed during the course of the patient's illness may have been due in part to circulatory changes, as it is well known that vomiting frequently accompanies dizziness, but the gastric catarrh observed was attributed to the local irritant action of the drug.

Koppe<sup>7</sup> investigated the actions of digitoxin upon animals and himself, and the actions of other digitalis bodies upon animals, and his conclusions will be considered somewhat more extensively than those of others because they have undoubtedly exerted great influence upon the opinions of later writers on the subject.

Koppe worked in the laboratory of Schmiedeberg, where Harnack had but recently (1874) studied the actions of apomorphine, which induces emesis by its central action alone.

Koppe states that digitoxin administered to dogs caused emesis

<sup>3</sup> *Commentaires théor.*, etc., Paris, 1874.

<sup>4</sup> *Principles of Therapeutics*, 1881, p. 155.

<sup>5</sup> *Compt. rend. Soc. de Biol.*, 1874, 6, S. 1, p. 297.

<sup>6</sup> *Deutsche mil. aerztliche Zeitsch.*, 1875, pp. 525; 636.

<sup>7</sup> *Arch. exp. Path. u. Pharm.*, 1875, iii, p. 274.

promptly. When the digitoxin was injected subcutaneously it caused phlegmonous inflammation, but digitalin and digitalein did not, the probable explanation being that their greater solubility permitted of their more rapid absorption and disappearance from the point where injected.

He states: "In a certain sense the emesis and diarrhea can be attributed to the local action, as the former probably is not of central origin."<sup>8</sup>

But while he speaks in such uncertain terms concerning the participation of a direct action on the vomiting center in the production of emesis, he also makes the following unqualified statement concerning digitoxin, digitalin and digitalein: "A direct influence of these poisons on the central nervous system is excluded in animals, and cannot be observed in man."<sup>9</sup>

Schmiedeberg<sup>10</sup> gives a critical and historical review of the literature of the digitalis bodies, and calls attention to the possibility of their local action on the stomach and intestines, with nausea and vomiting, even with therapeutic doses, but he directs attention to the presence of picrotoxin-like bodies in digitalis.

Haynes<sup>11</sup> used decerebrate cats in his studies designed to show the direct action of these agents on the gastric mucous membrane. He found that digitalis exerted a corrosive action, but it should be remembered that Haynes used massive doses in these experiments, and that the results so obtained are not at all applicable to therapeutic doses in man.

Strophanthus, or strophanthin, the digitalin of Schmiedeberg and Killiani, Nativelle's digitaline, or digitoxin, and ouabain differ more or less in their actions from digitalis after their oral administration.

Reports have appeared in the literature from time to time that one or the other of these substances lacks the direct gastro-in-

<sup>8</sup> "In gewissem Sinne kann man auch das Erbrechen und die Durchfaelle zu den Lokalerscheinungen rechnen, da auch das erstere wahrscheinlich nicht centralen Ursprungs ist."

<sup>9</sup> "Ein directer Einfluss dieser Gifte auf das centrale Nervensystem liess sich an Thieren ausschliessen und konnte am Menschen nicht beobachtet werden."

<sup>10</sup> Arch. exp. Path. u. Pharm., 1882, xvi, p. 149.

<sup>11</sup> Biochem. Jour., 1906, i. p. 62.

testinal irritant action which digitalis is said to possess, or that such substances exhibit the action to a much less degree.

Fraser<sup>12</sup> said that the pharmacology of strophanthin is very simple, the drug being a muscle poison, and that sickness and gastro-intestinal symptoms are less common after the use of strophanthus than after digitalis.

Essentially similar views have been expressed concerning the gastro-intestinal action of strophanthus, or strophanthin, by numerous clinical observers, among whom we will mention Pins<sup>13</sup> and quite recently by Cushny,<sup>14</sup> but this view was not accepted universally, and Fraenkel<sup>15</sup> records severe disturbances of digestion following its use.

Haynes<sup>16</sup> stated that strophanthus was much less irritant than digitalis to the gastric mucous membrane, and it must be remembered that he used massive doses, as previously stated.

Rosenbusch<sup>17</sup> observed nausea and vomiting in man after the subcutaneous injection of 2 mg. of amorphous strophanthin, while the tincture of strophanthus administered by the mouth was borne very well.

Rosenbusch did not draw any conclusions concerning the seat of the emetic action of strophanthus and strophanthin, and he appears to have been unaware of the fact that these bodies are qualitatively similar in their action, or that their absorption from the gastro-intestinal tract is slow and uncertain.

Gley and Lapicque<sup>18</sup> state that contrary to the prevailing opinion, strophanthus acts on the medulla, as shown by the fact that acceleration of respiration precedes the cardiac action; and Gley<sup>19</sup> concludes that ouabain and strophanthin act on the medulla as shown by the emesis and the respiratory effects which they induce.

<sup>12</sup> Brit. Med. Jour., 1885, ii, p. 904.

<sup>13</sup> Therap. Monats., 1887, p. 209.

<sup>14</sup> Brit. Med. Jour., Sept. 21, 1912.

<sup>15</sup> Therap. Monats., 1888, p. 75.

<sup>16</sup> Loc. cit.

<sup>17</sup> Berl. klin. Woch., 1888, p. 128.

<sup>18</sup> Compt. rend. Soc. Biol., 8, S. 4, 1887, p. 421.

<sup>19</sup> Compt. rend. de l'Acad. de Sc., Paris, 1888, cviii, p. 348.

Gunther<sup>20</sup> states that strophanthus exerts a specific action on the vomiting center, the conclusion being based upon results obtained by injecting massive doses of tincture of strophanthus into dogs subcutaneously and observing that emesis occurred even after the vagi had been cut. He believes that the local gastric effects due to excretion into the stomach are excluded by the fact that emesis followed within a minute after the injection.

The fixed oil in strophanthus, and that in digitalis have been considered as the cause of the gastric disturbance by some, but this view has never received support worthy of consideration.

Kiliani<sup>21</sup> states that Boehm found that digitonin caused direct injury to the gastric mucous membrane in the dog when enormous doses were used, but according to Kiliani<sup>22</sup> digitalis contains at most but the merest traces of digitonin, hence that constituent cannot be concerned in the emetic action of therapeutic doses of digitalis.

Dale and Laidlaw<sup>23</sup> believe that the local irritation of the stomach following therapeutic doses of apocynum by the mouth is to be attributed to some other principles than that upon which the cardiac action depends, but they conclude that the emesis and diarrhea which follow the subcutaneous injection of the principle having the cardiac action are due either to the central action, or, more probably, to a direct action on the smooth muscles of the stomach and intestines.<sup>24</sup>

An argument which is frequently heard is that the irritant action of the digitalis bodies after subcutaneous injection, or application to the mucous membrane of certain areas, suffices to explain the gastro-intestinal disturbances which follow their therapeutic use by the mouth. That certain of the digitalis

<sup>20</sup> Therap. Monats., 1904, p. 285.

<sup>21</sup> Arch. der Pharm., cexxx, p. 260.

<sup>22</sup> Arch. der Pharm., cexliii, p. 7.

<sup>23</sup> Heart, 1909, i., p. 138.

<sup>24</sup> Numerous pharmaceutical preparations are exploited which are said to be free from the local gastro-intestinal action of digitalis, but we shall avoid a discussion of this phase of the question at this time as we have in course of preparation an article dealing with the subject of the comparative actions of the digitalis bodies.

principles are extremely irritant to the nasal mucous membrane is shown by the fact that we have been able to detect the irritant action of as little as one three-thousandth of a milligram of ouabain which had been triturated with sugar of milk and then insufflated into the nostril.

#### SIGNIFICANCE OF SEAT OF EMETIC ACTION

The literature relating to the question of the existence of a special center for emesis has been discussed by us<sup>25</sup> in connection with the investigation of the seat of the emetic action of apomorphine, and it is unnecessary to discuss it again in this place.

A critical examination of the literature at our disposal discloses the fact that no convincing arguments have been advanced concerning the seat of the emetic action of any of the digitalis bodies, or that the mechanism is the same with the different members of the group, but that the statements commonly found in text-books concerning this action are based upon slight evidence.

It must be said, however, that there is strong presumptive evidence that very large doses of *strophanthus*—not such as are used therapeutically—may induce emesis through the direct action on the vomiting center, and there is equally strong, or even stronger, evidence that very large doses—but not such as are used therapeutically—of other members of the group are capable of causing emesis through the direct local irritant action on the mucous membrane of the stomach.

Both of these observations leave the question of the emetic action of the digitalis bodies unanswered insofar as it concerns the clinician, who is not interested primarily in the actions of enormous amounts of these drugs, but in the action of amounts comparable to those which he uses therapeutically.

It is obviously of the first importance to the clinician to know whether the emetic action is a purely local one, to be avoided or mitigated by appropriate means of administering the drugs or whether the nausea and emesis are of central origin, and therefore wholly independent of the mode of administration, but directly

<sup>25</sup> Eggleston and Hatcher, *Jour. Pharm. and Exp. Therap.*, iii, p. 551.



dependent upon the amount of the drug which enters the circulation in a given case.

If the emetic action is a purely central one we must seek to find which of the members of the group possess the least of the undesired actions in proportion to their cardiac activity.

There are several reasons which partly explain why this subject has not received sufficient attention at the hands of pharmacologists.

In the first place digitalis, the most popular member of the group, contains principles the nature of which is not understood, and not all of these principles are absorbable from the alimentary canal, hence, though emesis were shown to be of central origin after the intravenous administration of this drug, that would not at all disprove the contention that the local irritant action on the mucous membrane of the stomach is the cause of the emesis which follows the oral administration, since the mechanism of emesis may be quite different in the two cases.

Indeed, digitonin does appear to have two different modes of inducing emesis, for it exerts this action whether it be administered intravenously or by the stomach, and it is generally stated that it is not absorbable from the alimentary canal. If this belief concerning its non-absorbability is correct, it must act locally after the oral administration, but the rapidity with which it induces emesis after the intravenous administration leads one to suspect that direct stimulation of the vomiting center is then concerned.

The following facts have undoubtedly contributed to prevent the correct interpretation of these phenomena during studies of digitalis: Pharmacologic experiments involving operations on mammals are usually carried out under anesthesia which prevents emesis, and chloroform and ether, which are commonly used as anesthetics, frequently give rise to emesis when consciousness returns. In the next place, very large doses of digitalis are generally used in pharmacologic experiments with the drug, and such doses, however administered, induce emesis promptly in normal cats and dogs.

While these, and other more or less related, circumstances have contributed to prevent the proper investigation of the question, we must suppose that pharmacologists have believed digitalis to be extremely irritant to the mucous membrane of the stomach because small amounts are extraordinarily irritant to subcutaneous tissues and to the mucous membranes of certain areas, and also because large amounts have been shown to be irritant, or even corrosive, to the gastric mucous membrane.

Our attention has been directed to the possibility that the emetic action of moderate amounts of the different digitalis bodies might be of central origin, at least in part, at various times during the past few years.

One of the most striking of the facts observed which points to the action of digitalis on the vomiting center is that a smaller dose suffices by intravenous or subcutaneous injection than by stomach to induce nausea and vomiting.<sup>26</sup>

The difference between the effective doses by oral and intravenous administration is very much greater with certain members of the group than with others, and digitalis and digitoxin are the only ones which we have found to be capable of inducing emesis in the majority of cases with nearly so small a dose administered by the mouth as that required by intravenous injection. The reason for this activity by oral administration is to be found in the greater absorbability of these two drugs than of other members of the group.

Closely connected with the preceding observation is the fact that emesis may be induced far more rapidly by moderate doses of the digitalis bodies administered intravenously than by similar, or somewhat larger, doses given by the mouth.

Emesis follows the intravenous administration of moderate doses of the digitalis bodies so promptly that not more than the merest traces of the drugs can be excreted into the gastro-intestinal canal before this action is induced, and emesis may be induced within two minutes after the intravenous injection of a large dose of digitoxin.

<sup>26</sup> Hatcher, *Am. J. Phys.*, 1909, xxiii, p. 310.

We were inclined at first to accept the view quite generally held that emesis following promptly after the intravenous injection of a drug afforded strong evidence in support of the contention that it acted directly on the vomiting center.

We believe, however, that slow excretion of a drug is not necessarily incompatible with the rapid production of emesis through its local irritant action while in process of excretion.

It is quite conceivable that a drug may be very much more actively emetic after its intravenous injection than after its oral administration, even though it may act upon the reflex mechanism in the wall of the stomach in both instances, for it is possible that the drug may be brought through the circulation into intimate contact with the structure in the wall of the stomach which is concerned in the vomiting reflex very much more rapidly than it can by traversing the intervening part of the wall of the stomach after the oral administration.

It is suggestive that the period which elapses between the intravenous administration of a moderately large dose of digitoxin or ouabain and the occurrence of emesis—about two minutes—corresponds fairly closely with the interval which elapses between the intravenous administration of certain of these drugs and their disappearance from the blood stream, as shown in experiments performed in this laboratory.

We know practically nothing of the distribution of the drugs of this group in the animal organism during the interval which follows their disappearance from the blood stream and before their excretion, but we do know that at least one of these bodies, ouabain, is excreted into the intestines of the rat after its subcutaneous injection.<sup>27</sup>

Another observation made independently of the present research also pointed to the probability of the central emetic action of these bodies, but with less force, perhaps, than the facts previously cited.

During studies of absorption of the digitalis bodies we observed that frequently emesis did not occur after the oral administra-

<sup>27</sup> Hatcher, *loc. cit.*

tion of amounts of these drugs equal to several times the fatal dose by vein. In such cases subsequent biologic tests showed that little or none of the drug so administered had been absorbed into the blood stream.<sup>28</sup> In those cases where emesis did follow the oral administration of these drugs, and in which the amount absorbed was determined it was found that the amount so absorbed was roughly equal to that which is required by intravenous administration to cause emesis.

#### PRELIMINARY EXPERIMENTS

The observations which served to fix our attention on the vomiting center as the possible seat of the emetic action of the digitalis bodies afforded no trustworthy evidence concerning the nature of this action, and in planning the investigation of the problem we decided to employ methods similar to those which had been used to demonstrate the seat of the emetic action of apomorphine, because we believed that more convincing evidence had been submitted in support of its central action than in the case of any other drug.

The first step in the pursuance of this plan was to test the action of some of the digitalis bodies on animals in which the vagi had been cut in order to sever the path by which reflexes from the mucous membrane of the stomach are supposed to be carried to the vomiting center in the medulla.

This simple procedure of cutting the vagi does not suffice to exclude reflexes through other possible paths from the gastrointestinal tract to the vomiting center, nevertheless, the prompt appearance of emesis following the intravenous injection of a moderate amount of a drug after cutting the vagi would afford corroborative evidence of its central action.

With this object in view three experiments were performed, the results of which were very unsatisfactory. Emesis actually occurred in two of the three experiments, but it was so long delayed in both cases as to suggest the possibility of some other mechanism than the direct action on the vomiting center.

<sup>28</sup> The method of testing the degree of absorption of these bodies has been described by one of us. Jour. Am. Med. Assoc., 1910, lv, p, 746.

The next step in the investigation consisted in the attempt to exclude the injected drug from the stomach by tying the vessels which supply blood to that organ. Six cats were used for these experiments.

The superior mesenteric artery and the coeliac axis were tied in every case, and four of the animals received ouabain subcutaneously, and two were used as controls. All of the animals vomited and no conclusions could be drawn concerning the seat of the emetic action of the drug.

A cannula was inserted into the aorta after the death of one of the animals and a solution containing 2 per cent of sodium chloride was perfused through the vessels. A slight oozing from the stomach indicated that the circulation to that organ had not been cut off completely though the ligatures on the arteries had been tied effectively.

Two factors which operated to prevent the success of these experiments require mention. Emesis sometimes follows the administration of ether and chloroform to cats, and on the other hand, the depression resulting from such severe operative procedure often interferes with the emetic action of any drug.

The work was interrupted at about this time while we sought for an improved technic, and in the meantime the investigation of the emetic action of apomorphine was undertaken with the results described in our previous paper.

#### EXPERIMENTS ON EVISCERATED DOGS

The experiments with apomorphine on eviscerated dogs proved so satisfactory, and appeared to settle the question concerning the seat of its emetic action so conclusively, that we abandoned the plan of investigating the emetic action of the digitalis bodies by other methods, and proceeded with the experiments similar to those described in our previous paper except insofar as the slight modification which the use of the digitalis bodies in the place of apomorphine required.

The emetic action of many of the digitalis bodies is by no means so prominent and early as it is with apomorphine, and it is not equally prominent with all of the members of the group, still, it

may be said that emesis is a fairly constant symptom with sufficient doses of all of these agents, and it is rarely absent when suitable doses are administered to the normal cat or dog.

The technic of the operation for the removal of the gastro-intestinal tract in dogs has been described in our previous paper.

The protocol of a single experiment will be included here and the results of the experiments with the digitalis bodies on eviscerated dogs will be given in the tables.

*April 9, 1912. Mongrel, female dog; weight 10.3 kg.:*

12.18 p.m. Chloroform anesthesia.  
 12.21 p.m. Operation begun.  
 12.25 p.m. Chloroform administration discontinued.  
 12.44 p.m. Digitalis injected intravenously.  
 12.45 p.m. Salivation pronounced.  
 12.47 p.m. Emesis.  
 12.49 p.m. Urinated, defecated.  
 12.50 p.m. Experiment ended; animal killed with chloroform.

TABLE 1  
*Showing the emetic action of digitalis by vein on eviscerated dogs*  
*First series*

TIME BETWEEN OPERATION AND INJECTION	DOSE PER KILOGRAM	RESULTS
<i>minutes</i>	<i>mg.</i>	
59	33	Nausea only
41	72	Emesis
33	90	Nausea only
83	97	Emesis
59	83	Emesis
54	88	Much depressed; no emesis
50	133	Emesis
42	?	Nausea only
39	100	Emesis
<i>Second series</i>		
82	100	Emesis in 8 minutes
46	100	Emesis in 5 minutes
17	100*	Emesis in 3 minutes
10	100*	Emesis in 8 minutes
15	100*	Emesis in 10 minutes

\* An infusion of undetermined strength was used in these three experiments, but the dose was less than that given, because the infusion used did not represent the activity of the drug fully.

We have shown in our previous paper that eviscerated dogs seldom vomit as a result of the operation or the chloroform used, hence controls were not considered necessary in the present series of experiments, but the intervals following the operation and preceding the administration of the drug in all but the last three experiments of table 1, will serve as a control should one deem that necessary; particularly is this evident when one observes the prompt appearance of emesis in the experiments in the second series.

Tincture of digitalis of known activity was used in all of the experiments in the first series, and in two of those in the second series; in these the injections were made slowly and continuously until nausea was produced, or (in one experiment only) until the extreme depression of the animal indicated the hopelessness of further attempts to induce emesis.

Signs of nausea in the cat and dog consist in sudden and profuse salivation, with licking of the lips and swallowing movements, and are as unmistakable as the vomiting movements themselves.

Some of the partial failures in the earlier experiments with digitalis on eviscerated animals resulted from having the dogs anesthetised lightly. We believed that recovery would be more complete if the degree of anesthesia were just sufficient to permit of the operation being done without pain but experience soon convinced us that fairly deep anesthesia was the best for the purpose, and the animals returned to a more nearly normal condition in such cases than in those in which the anesthesia was either too light or too deep.

It will be seen that there was but one complete failure in the entire list of fourteen experiments with digitalis, since nausea was unmistakably present in all the other cases.

In the experiment in which great depression followed the operation the digitalis was injected with little hope of a successful outcome, and with our present experience such an animal would be discarded without attempting to induce emesis, for one might almost as well expect to cause emesis during profound chloroform narcosis as under such conditions.

The animals used in the last three experiments tabulated were in such good condition after the removal of the gastro-intestinal

tract that the injections were made almost immediately, and in every case emesis occurred promptly.

The doses of digitalis are expressed in milligrams of the powdered drug (represented by the tincture) per kilogram of weight of the animal, and the figures are also roughly percentages of the fatal dose by vein, since the fatal intravenous dose of this specimen of digitalis is not far from 100 mg. per kilogram of weight (more nearly 125 mg. per kilogram), but dogs manifest greater individual differences than cats in susceptibility to the digitalis bodies.

The results obtained with several of the other members of the digitalis group with similar experiments on eviscerated dogs are given in table 2.

TABLE 2  
*Showing the emetic action of various digitalis bodies on eviscerated dogs with intravenous administration*

DRUG	DOSE PER KILOGRAM	RESULTS
	<i>mg.</i>	
Digitoxin.....	0.50	Emesis in 7 minutes
Digitoxin.....	{ 0.50	Death, without emesis, 4 minutes after second dose
	{ 0.25	
True digitalin.....	1.50	Emesis in 2 minutes
Ouabain.....	0.10	Emesis in 5 minutes
Strophanthus.....	2.50	Emesis in 1 minute and 40 seconds
Amorphous strophanthin...	{ 0.10	Emesis in 2 minutes and 30 seconds after second dose
	{ 0.025	
Adonis.....	{ 65.00 } (about)	Emesis in 5 minutes

The doses administered in all of the experiments in table 2 with the exception of that in which two doses of digitoxin were administered, were slightly below the fatal.

Reference to the table shows that six of the seven animals vomited. The remaining one, which received the fatal dose of digitoxin in two portions, appears to have been refractory to the emetic action of the drug. This refractory condition is seen occasionally in normal animals, and even apomorphine fails occasionally to induce emesis after intravenous administration, for example, in those cases where the required amount is exceeded at a single injection.



To recapitulate the results, sixteen of the twenty-one eviscerated animals vomited after the injection of the digitalis bodies, and three of the others showed severe nausea. The first animal received an insufficient dose; one appears to have been refractory toward the drug; and one was so greatly depressed after the operation that it should not have been used for the emetic test.

It is not at all remarkable that a few of these animals failed to vomit, and in fact, a certain percentage of normal animals will die without vomiting after the injection of large doses of the digitalis bodies. The surprising thing is that so many of them passed through an operation of this character without sustaining injuries which would have prevented the success of the experiments.

No attempt was made during these experiments to determine the minimum emetic dose of any of the digitalis bodies because we were interested mainly in demonstrating that these substances are capable of inducing emesis in dogs after the removal of the gastro-intestinal tract<sup>29</sup> but the method of administering the drugs which was used in the experiments with digitalis enabled us to avoid the use of doses greatly in excess of the minimal which would prove promptly effective; there is little doubt, however, that somewhat smaller doses would have produced emesis after a longer interval in some cases.

Reference to the experiment with ouabain showed that this animal vomited in five minutes after receiving 0.1 mg. of ouabain per kilogram of body weight. This is only about one-tenth as much in proportion to the weight of the animal as Hochheim administered to his patient by the mouth on each of two successive days.<sup>30</sup> The total dose in that case having been 75 mg. per day.

Another of the eviscerated dogs vomited in one minute and forty seconds after receiving 2.5 mg. of strophanthus (or, to be more exact, 25 mg. of the tincture) per kilogram of weight. This is equal to only about one-fifth as much in proportion to the weight as that taken by a patient on each of two successive days without perceptible effect.<sup>31</sup>

<sup>29</sup> We employ the word "emesis" in this seemingly paradoxical way because the phenomenon is indistinguishable from that observed in a perfectly normal animal.

<sup>30</sup> Loc. cit.

<sup>31</sup> Hatcher, loc. cit.

## RESULTS OF ADMINISTERING THE DIGITALIS BODIES ORALLY

We have frequently administered large amounts of drugs of this group to cats by the mouth, and a few examples of the results thus obtained will suffice to show how slight is the direct action of these substances on the mucous membrane of the stomach.

One of the cats received an amount of convallaria equal to one hundred times the fatal dose by vein. The animal was observed for an hour and thirty-five minutes; during that period emesis did not occur, and a second dose equal to sixty times the fatal dose by vein was then given. This was followed by emesis.

Another cat received an amount of squill equal to ten times the fatal dose by vein, but no effect could be perceived.

Emesis occurred in only eight experiments in a series of eighteen in which ouabain was administered to cats by the mouth in amounts varying from two to ten times the fatal dose by vein, and curiously, the smallest of these doses caused emesis, but in two of the experiments, in each of which the amount was equal to ten times the fatal dose by vein, emesis did not occur.

It is obvious that comparatively little of the convallaria, squill and ouabain was absorbed in those experiments, since death would have followed promptly in that event.

In three experiments the animals severally received by the mouth an amount of ouabain equal to five times the fatal dose by vein. Emesis did not occur and the subsequent biologic test failed to show that any of the ouabain had been absorbed.

Analogous results were obtained with amorphous strophanthin and it is unnecessary to discuss them in detail.

Digitoxin was administered orally to seven cats in amounts varying from the equivalent of half the fatal dose by vein to six times as much. Emesis occurred in every case, and biologic tests showed that the amounts which were absorbed into the circulation varied from 16 to 75 per cent of the fatal dose by vein.

The amounts of digitoxin thus shown to have been absorbed correspond fairly well with those which we have recently found necessary by vein to cause emesis.

Results were obtained after the oral administration of digitalis to cats which are strictly comparable to those just mentioned with digitoxin, and these are to be explained in the same way, biologic tests having shown that enough of the digitalis had been absorbed to account for the emesis in every case.

Strophanthus, strophanthin and ouabain are generally supposed to be much less irritant than digitoxin to the mucous membrane of the stomach<sup>32</sup> but the results just mentioned with regard to convallaria, squill, ouabain, digitoxin and digitalis would seem to indicate that differences in their emetic action after oral administration are to be attributed to differences in absorbability rather than to any differences in their direct irritant action on the gastric mucous membrane.

The results of these experiments serve to show that the mucous membrane of the cat's stomach is tolerant towards certain of the digitalis bodies which are not absorbed readily from that organ, but the following may be cited as an example of even greater tolerance on the part of a mucous membrane toward one of these bodies, the absorption of which it resists completely.

Each of several rats received about 100 mg. of ouabain mixed with a little cracker dust. This was eaten readily without the manifestation of any symptoms of irritation of the gastric mucous membrane.

The tolerance of these animals towards ouabain is remarkable, and such doses must be regarded as stupendous for such small animals.

One appreciates the differences in susceptibility of different mucous membranes better perhaps, when he remembers that the amounts tolerated by those animals without visible manifestations of discomfort were many thousands of times greater than those which suffice to cause distinct discomfort when applied to the nasal mucous membrane in man.

We believe that the explanation of the wide difference in the susceptibility of the mucous membranes of different regions to the same agent is to be sought in more than one direction, and that

<sup>32</sup> Cf. Dixon, loc. cit.

the physical properties of the substance and the functions of the several mucous membranes are of prime importance in this connection.

Digitoxin is wholly insoluble in water and it cannot be brought instantly into intimate contact with the sensory nervous mechanism of the nasal mucous membrane, for the function of this surface is not that of absorption primarily, and we found that digitoxin is not so irritant as ouabain to this region, ouabain being quite soluble, and much more easily brought into intimate contact with the sensory nervous mechanism.

Absorption is a function of the mucous membrane of the stomach, and the exercise of this function does not require that the substance to be absorbed shall be readily soluble in water. On the other hand, the solubility of a substance in water does not insure its absorbability in the stomach. It has been found<sup>33</sup> that ouabain is not absorbed at all from the gastro-intestinal tract of the rat, and its absorption is slow and uncertain after the oral administration to man, as well as the cat and dog.

An illustration of this is had in the case of Hochheim's patient, previously mentioned, who received 75 mg. of ouabain by the mouth on each of two successive days; amounts which are certainly equal to many times the fatal dose for a man by the vein.

A similar resistance to the absorption of strophanthus has been noted in the case of the patient previously mentioned.

So far as our experiments extend they show that the absorbability and the emetic action of the digitalis bodies after the oral administration go in the same direction at least, if they do not go strictly parallel, whereas the solubility of these agents in water and their absorbability in the gastro-intestinal tract frequently go in opposite directions.

This might be stated somewhat differently as follows: Those digitalis bodies which we have tested and found to be actively emetic after the oral administration have also been found to be fairly rapidly absorbable from the alimentary tract; but on the other hand, those digitalis bodies which were not actively emetic

<sup>33</sup> Hatcher, loc. cit.

after their oral administration, were absorbed slowly, or not at all, from the gastro-intestinal tract.

Digitoxin is quite insoluble in water, as previously stated, but is absorbed fairly rapidly from the alimentary tract of the dog and cat, hence it is actively emetic when administered by the mouth. Ouabain and amorphous strophanthin are very soluble in water, but they are absorbed slowly as a rule from the gastro-intestinal tract and are not actively emetic after their oral administration except in those rare cases where absorption follows fairly rapidly.

It is necessary to lay stress upon these facts because one finds in the literature many statements which appear to be based upon seemingly obvious logic rather than upon experimental evidence, and one is tempted to say of the digitalis bodies that the seemingly obvious is almost certain to be incorrect.

#### SUMMARY

Emesis is one of the most common symptoms of poisoning with the members of the digitalis group.

All of the digitalis bodies are capable of causing emesis, but the different members of the group apparently show much greater differences in emetic, than in cardiac, activity with a given species of animal.

The view generally held that this emetic action occurring with the clinical use of the digitalis bodies is due wholly or chiefly to the direct irritant action on the mucous membrane of the stomach, is based upon the fact that all of the drugs of the group exert an irritant action upon certain mucous membranes and upon subcutaneous tissues, and upon the results obtained by introducing into the stomach amounts much greater than those used clinically. Such massive doses of the digitalis bodies may cause emesis by their direct irritant action on the gastric mucous membrane but we are not concerned at present with that phase of the question.

The intensity of this irritation of the mucous membranes probably depends largely upon the solubility, and other physical properties, of the agent, and upon the function of the mucous membrane, particularly, its capacity for absorbing the drug, or resisting its absorption.

The absorbability of the different digitalis bodies from the gastro-intestinal tract of the cat and dog, and their emetic activity after oral administration to those animals go in the same direction but not strictly parallel.

Ouabain apparently does not irritate the mucous membrane of the rat's gastro-intestinal canal, from which it is not absorbed even after the administration of very large doses.

We know little of the distribution of the digitalis bodies in the animal organism during the period after they leave the blood stream and before they are excreted, hence there is no sufficient basis for the argument that a drug of this group which induces emesis promptly after its intravenous administration, and only after a much longer interval following its oral use, must therefore be capable of acting directly upon the vomiting center in the medulla.

It is quite true, however, that such emetic action after the intravenous administration does serve to direct attention to the probability of its being of central origin in those cases.

All of the digitalis bodies which we have tested in this way induce emesis more promptly when they are injected intravenously than when they are administered by the mouth, and smaller doses are required by the vein than by the mouth for this purpose.

No conclusive evidence has been afforded hitherto concerning the seat of the emetic action of moderate doses of any of the digitalis bodies.

The amounts of ouabain and strophanthus which are required by the vein to induce emesis in eviscerated dogs are very much less actually, as well as relatively in proportion to their weight, than are the amounts of these drugs which have been used clinically by the mouth, and in some cases without perceptible effects.

Our experiments on eviscerated dogs prove conclusively that those digitalis bodies which we employed in this way are capable of inducing emesis, or nausea and vomiting movements, in dogs without the participation of the action on the gastro-intestinal tract.

The results obtained after the oral administration of strophanthus and ouabain to man and the similarity of the behavior of

the digitalis bodies in man, to that observed in the cat and dog, leave no ground for supposing that the mechanism of the emetic action in man is in any way different from that seen in those animals, hence we are led irresistibly to the conclusion that the emesis sometimes seen in man after the oral administration of therapeutic doses of digitalis bodies is due mainly, if not exclusively, to their action on the vomiting center in the medulla.

#### CONCLUSIONS

1. The digitalis bodies vary widely in the degree of the direct irritant action which they exert upon a given mucous membrane; and the mucous membranes of different areas in the same animal, or those of corresponding areas in different animals, vary even more widely in the degree of their susceptibility to the irritant action of any given member of the digitalis group, so that one of these agents may be intensely irritant to the mucous membrane of one area in a certain animal, and yet without appreciable influence upon the mucous membrane of another area in the same animal.

2. Ouabain is intensely irritant to the mucous membrane of the nose, and to the subcutaneous tissues in man, but it is borne in large amounts without signs of gastro-intestinal irritation when administered to rats by the mouth, and this drug may fail to induce emesis in man, as well as in the cat and dog, after the oral administration of considerable amounts unless absorption occurs.

3. All of the digitalis bodies which we have tested induce emesis in cats more promptly and in smaller doses after intravenous administration than when they are given by stomach; and digitalis, digitoxin, true digitalin, strophanthus, amorphous strophanthin, ouabain and adonis (and probably other members of the group), induce emesis promptly after their intravenous injection into dogs from which the gastro-intestinal tract from the esophagus to the lower part of the rectum has been removed, leading irresistibly to the conclusion that the emetic actions of these drugs is exerted upon the vomiting center in such cases.

The purgative action is also obviously of central origin.

4. The doses of the digitalis bodies required to produce emesis in eviscerated dogs are strictly comparable to those used therapeutically, and the average emetic dose of ouabain or strophanthus for eviscerated dogs is actually far less in proportion to the weight than the doses of those drugs which have been administered to patients by the mouth within the period of one day.

5. In the face of the evidence existing that the emetic action of therapeutic doses of the digitalis bodies is exerted on the vomiting center in man the burden of proof to the contrary certainly rests upon those who would contend that the emetic action is of peripheral origin.

We are indebted to Mr. M. I. Smith of the Senior Class, for assistance in some of the preliminary experiments in this research.



## THE PHARMACOLOGICAL ACTION OF CORIAMYRTIN

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Coriamyrtin, the active principle of *Coriaria myrtifolia* (L.), an ornamental shrub indigenous to the south of France and other countries bordering the Mediterranean, was first isolated and investigated by Riban.<sup>1</sup> He found it to be a crystalline glucoside melting at 220°C. (corr.) and soluble in 70 parts of water or 50 parts of alcohol at 22°C. The coriamyrtin supplied by Merck, which I had occasion to investigate in connection with another research, melts at 223°C.<sup>2</sup> and is very slightly soluble in water, one part requiring 2500 parts of water at 12°C. This difference in solubility suggests that the two coriamyrtins are not identical, a conclusion which seems to be supported by what appears to be a difference in the ease with which they undergo hydrolysis. Both coriamyrtins produce the same symptoms in animals but owing to Riban having used large toxic doses in his experiments it is impossible to say if any quantitative difference exists between them pharmacologically. Since the appearance of Riban's paper coriamyrtin has been investigated, for the most part for special purposes, by Perrier, Köppen, Zutz, Gottlieb, Harnack, and Fitchett and Malcolm, but unfortunately no physical characters of the coriamyrtin used are given in their papers.

### GENERAL EFFECTS OF CORIAMYRTIN (MERCK)

My experiments on the general effects of coriamyrtin were limited to rabbits, guinea-pigs, and frogs, and, with one exception, to hypodermic administration.

<sup>1</sup> Recherches expérimentales sur le principe toxique du redoul, Paris, 1863.

<sup>2</sup> Merck (Annual Report for year 1898, p. 52) gives as the melting point 228° to 229° C.

*Rabbits.*

After a hypodermic injection of 1 mg. per kilogram body weight to full grown or nearly full grown rabbits there appears within five minutes a notable increase in the frequency of the respiration which is followed a few minutes later by retraction of the ears, and even of the head, and twitchings about the eyes, mouth and nose. Noddings and tremors of the whole head develop and are quickly followed by coarser tremors over the whole of the body which are superseded by slight clonic movements. These are usually of short duration and during their occurrence, but sometimes independently, the limbs become straddled and the animal gradually sinks on the table. At this time there is often some salivation, the corneal reflex is sluggish, the ear vessels are somewhat dilated, and the respiration, although faster than normal, is less rapid than at first. After a few minutes clonic movements reappear and become more powerful. At first they are somewhat irregular and may be interrupted by tonic spasms (opisthotonus): later, when the animal has become unconscious and fallen on its side, they assume the character of running movements, which with varying severity, may continue for hours. The corneal reflex is commonly absent during this period. The animal may recover or may die from exhaustion. If chloroformed the clonic movements quickly cease, the retraction of the head, which is invariably present, being usually the last convulsive state to go.

After 0.5 mg. per kilogram body weight hypodermically similar but less marked symptoms are observed. The respirations increase in frequency and depth, the animal assumes a straddled position and tends to sink on to the table, and tremors of the head appear. Later, movements of the fore part of the body occur. Usually all symptoms have disappeared forty minutes after the injection.

After 0.3 mg. per kilogram body weight an increase in the frequency of the respiration, a stretching forwards and unsteadiness of the fore part of the body, and a sinking down on to the table are noticed. The animal is apparently normal twenty minutes after the administration.

The only symptoms observed after the injection of 0.13 mg. per kilogram body weight were slight increase in the frequency of respiration and slight and transient tremors of the head.

The general effect of coriamyrtin on rabbits has been described by Riban, Köppen, and Zutz. Riban<sup>3</sup> administered the coriamyrtin isolated by him in doses of 0.08 gram by the mouth to fasting animals. Movements of the head commenced, in the three protocols given, in ten, five, and twenty minutes, and clonic convulsions with occasional tonic spasms continued almost uninterruptedly until death occurred eighty-five, forty-five, and twenty-five minutes respectively after the administrations. In one case 0.02 gram of powdered coriamyrtin was applied under the skin. Convulsions commenced five minutes after the administration and death occurred twenty minutes later. Köppen<sup>4</sup> observed slight convulsions after the injection hypodermically of 1 mg., but not after the injection of 0.5 mg. coriamyrtin to a medium sized rabbit. The smaller dose, however, produced in one case transient shaking (*Erschütterung*) of the whole body, an effect which he associates, probably correctly, with the greater frequency of respiration. Zutz<sup>5</sup> noticed restlessness and pushing out of the fore limbs after two doses of 0.5 mg. hypodermically at an interval of forty-one minutes, and after 1 mg. increased respiration, tremors, and convulsive turning of the head. After the injection hypodermically of 1.5 mg. to a rabbit weighing 2090 gram powerful clonic convulsions occurred twenty-five minutes later.

#### *Guinea-pigs*

No dose larger than 0.3 mg. per kilogram hypodermically was administered to these animals. The symptoms were similar to those observed in rabbits to which the same dose had been given, but in one case after 0.3 mg. per kilogram the respiration was noted to be slower although deeper than normal. Zutz<sup>6</sup> injected

<sup>3</sup> Loc. cit., pp. 65-70.

<sup>4</sup> Arch. f. exp. Path. u. Pharm., xxix, p. 343, 1892.

<sup>5</sup> Ibid., xxxviii, p. 411, 1897.

<sup>6</sup> Loc. cit., p. 412.

hypodermically 1 mg. into a guinea-pig weighing 410 grams; clonic spasms appeared in three minutes and death occurred twenty-eight minutes after the administration.

### *Frogs*

After the injection of 0.001 mg. per gram body weight into the dorsal lymph sac, symptoms may not appear for three quarters of an hour. The earliest are an increase in the frequency of the respirations, slight muscular stiffness and incoördination with consequent sluggishness of movement, and sinking on to the table. For long periods the animal lies on the venter with the limbs straddled and seems paralysed, but if placed on its back it recovers its position and usually exhibits slight tonic (opisthotonus) and clonic spasms. These may also be excited by attempts to spring, and occasionally an emprosthotonic position is assumed without apparent cause. The corneal reflex is present throughout the intoxication. The animal recovers after a few hours, but some stiffness of movement may still be seen twenty hours after the injection.

After 0.0025 mg. per gram body weight symptoms appear in about twelve minutes, and after 0.005 mg. per gram in about seven minutes after the administration. The animal sinks completely on the table and, later, occasionally pushes itself along in this position. Tonic spasms, usually limited in the case of the smaller dose to the fore part of the body (retraction of head, etc.) occur at short intervals.

Much larger doses induce symptoms very rapidly. After 0.033 mg. per gram body weight the animal sinks on the table two and a half minutes after the injection and enters into convulsions, often with a preliminary cry, about half a minute later. After 0.066 mg. per gram the animal is laid on the venter within one and a half minutes and almost immediately afterwards spasms appear. These are for the most part tetanic (opisthotonus) and of relatively short duration. During the first five minutes they occur frequently, then the intervals between the convulsions, which may now affect mainly and even be confined

to the hind legs, become longer, and after the lapse of half an hour spasms are relatively rare. In the intervals between the convulsions, and after they have ceased, the animal lies flaccid, but occasionally futile attempts to push itself along are made. This paralytic condition may continue for several hours.

The effect of coriamyrtin on the frog has been described by Riban, Perrier, and Köppen. The symptoms observed agree, in general, with those already described, but a strict comparison as regards dose at least, is not possible. Riban,<sup>7</sup> in the two frogs he experimented with, applied 0.015 gram of powdered coriamyrtin under the skin of the back. In both frogs tetanic convulsions appeared in ten minutes. Perrier<sup>8</sup> describes the effect of 0.5 mg. (probably on *Rana esculenta*). After a state of somnolence, convulsions followed ten minutes after the administration. He states that 0.2 mg. acts more powerfully than 0.5 mg. Köppen's<sup>9</sup> four protocols detail the action of 1 mg., apparently also on *Rana esculenta*. Convulsions appeared in eight, five and a half, six, and four minutes respectively.

*Effect of repeated doses of coriamyrtin on rabbits*

Coriamyrtin does not appear to be distinctly cumulative in its action. As already mentioned the effects of non-convulsive doses appear to pass quickly away, a rabbit being apparently normal forty minutes after a dose of 0.5 mg. per kilogram body weight, or twenty minutes after 0.3 mg. per kilogram body weight. There is slightly greater susceptibility to the drug for a short time longer, but if the intervals between the administrations be much greater than those mentioned, the effects produced by a given dose are fairly constant. Double the minimal convulsive dose can be given to rabbits without exciting convulsions if the administration is extended over three to four hours. The following protocol of an experiment illustrates this point. After each dose there was an increase in the frequency of the respirations with

<sup>7</sup> Loc. cit., p. 72.

<sup>8</sup> Arch. f. exp. Path. u. Pharm., iv, p. 204, 1875.

<sup>9</sup> Loc. cit., p. 341.

fine rapid nodding of the head, which seemed synchronous with the respiration, but for brevity these symptoms are omitted from and only the convulsive effects are mentioned in the protocol.

*Rabbit. Weight, 2300 grams*

TIME	DOSE OF CORIAMYRTIN	EFFECT
11.15	0.13 mg. per kg. subcutaneously	
11.51½	0.13 mg. per kg. subcutaneously	
12.23	0.13 mg. per kg. subcutaneously	
12.56	0.13 mg. per kg. subcutaneously	
1.15	0.13 mg. per kg. subcutaneously	
1.31	0.20 mg. per kg. subcutaneously	
1.50	0.20 mg. per kg. subcutaneously	
2.05	0.26 mg. per kg. subcutaneously	
2.07	0.26 mg. per kg. subcutaneously	Two twitches
2.15	0.39 mg. per kg. subcutaneously	
2.18	0.39 mg. per kg. subcutaneously	Twitch
2.20	0.39 mg. per kg. subcutaneously	Repeated slight twitchings, toes of fore limbs spreading
2.24	0.39 mg. per kg. subcutaneously	Eyelids twitching; animal moving about
2.26	0.39 mg. per kg. subcutaneously	Left ear twitching markedly
2.30	0.39 mg. per kg. subcutaneously	Coarse clonic movements of eyelids; head sinking on table
2.38	0.39 mg. per kg. subcutaneously	Appears as though clutching table; marked tone of limb muscles; slight abdominal twitch. Taken up; assumed normal posture when replaced
2.45	0.39 mg. per kg. subcutaneously	See text below.

Two and a half minutes after the last injection slight twitchings of the body often accompanied with rising on to the toes commenced and continued many times a minute until 2.54 when they became less frequent. Between these attacks the animal seemed normal. At 2.58 it was picked up and stroked several times without untoward effect, but on replacing it on the table and taking hold of one of its legs it entered into opisthotonus which lasted a minute and was followed by exhaustion. Consciousness was recovered two minutes later. At 3.04 head twitchings commenced and developed into a clonic convulsion extending over the whole body. No further convulsions occurred but forced movements

of the fore limbs were noticed for some time afterwards. The animal completely recovered. In this case 1.70 mg. per kilogram body weight was given over a period of three hours, more than half (1.0 mg. per kilogram) of which was administered during the last forty-five minutes, before distinct convulsive symptoms appeared, and these were of the mildest type.

An experiment in which repeated doses were given is described by Köppen.<sup>10</sup> He administered 0.8 mg. hypodermically in four doses of 0.2 mg., extending over a period of eighty minutes to a rabbit weighing 2490 grams and observed no effect beyond an increase in the frequency of the respirations. The total amount (0.32 mg. per kilogram), however, even if administered at one injection was too small to induce more than the slightest convulsive phenomena, nevertheless the experiment tends to show that cumulative effects do not readily occur.

This rapid and complete disappearance of the effects of a non-convulsive dose may be due to rapid excretion of the coriamyrtin from, or to its modification, probably decomposition, within the rabbit's body. The fact that in the frog a minimal effective dose takes a considerable time to induce symptoms points to the latter explanation.

#### ACTION ON THE RESPIRATION AND CIRCULATION

The three chief medullary centers are markedly stimulated by coriamyrtin. After non-convulsive doses, as Köppen long ago pointed out, the earliest and most obvious symptom is increase in the frequency of the respirations. *Pari passu* with this there is a diminution in the frequency of the pulse and a rise in the blood-pressure. During the earlier convulsions, and for a short time afterwards the respirations become fewer in number, but they may attain their former rapidity if the convulsion has not been too severe and if they are not interfered with by a subsequent convulsion. When the effect of the drug begins to pass away the frequency of the respirations and to a less extent of the heart-beats varies from time to time. The following protocol

<sup>10</sup> Loc. cit., p. 345.

shows the action of 0.5 mg. per kilogram body weight administered intravenously.

*Rabbit. Weight, 950 grams*

TIME	NUMBER OF RESPIRATIONS PER MINUTE	NUMBER OF HEART-BEATS PER MINUTE	REMARKS
<i>minutes</i>			
0	48	264	0.48 mg. coriamyrtin into marginal vein of ear
1½	204		
3		150	
4½	204	144	Head noddings marked
6½			Powerful clonic convulsions
8	84		
12	204		
15-17½			Convulsions
19	96		
21		204	
25	108	240	
44	60	264	
60	60	264	

After 0.3 mg. per kilogram body weight hypodermically the respirations rose from 72 per minute before to 180 per minute three minutes after the injection. Four minutes later they were again 72 per minute but they varied somewhat for seven minutes more, after which they were of normal frequency. An injection of 1 mg. per kilogram body weight hypodermically caused a rise in the frequency of the respirations from 108 per minute before to 312 per minute four minutes after the administration. The subsequent course, as far as the respiration was concerned, was interfered with by convulsions.

Köppen,<sup>11</sup> after the subcutaneous injection of 0.25 mg. per kilogram body weight observed in one case an increase in the frequency of the respirations from 128 to 152 per minute before to 216 per minute twenty-three minutes after the injection; and in another case a rise from 220-280 per minute before to 324 per minute fourteen minutes after the administration. After the injection of 1 mg. coriamyrtin subcutaneously to a rabbit (weight

<sup>11</sup> Loc. cit., p. 343.



not stated) an increase of respiratory frequency from 200 to 236 per minute to 332 per minute fourteen minutes after the injection occurred in one case, and a rise from 192 per minute to 356 per minute in a second case.

As will be seen from the protocol given above the pulse rate falls after the administration of coriamyrtin. This effect is due to stimulation of the cardio-inhibitory center. The vaso-motor center is also stimulated and the blood-pressure rises notwithstanding the fall in the frequency of the pulse. This is shown in the following protocol:

*Cat. Weight 3400 grams. Brain above Corpora Quadrigemina removed. Anesthetic stopped forty minutes before first injection.*

TIME	BLOOD-PRESSURE	PULSE BEATS IN 10 SECONDS	RESPIRATIONS IN 10 SECONDS	HEIGHT OF RESPIRATORY CURVE	REMARKS
<i>minutes</i>				<i>mm.</i>	
0	78	25	2.8	3.0	1 cc. 1/2000 coriamyrtin intravenously
1	82	17	2.0	4.0	
2	91	17	5.0	5.0	
4	92		12.0	7-8	
5	88	21	13.0	5.5	
7	91	22	7.5	3.0	
15	94	21	7.5	4.0	1 cc. 1/2000 coriamyrtin intravenously
16					
16½	128	15			
17	122	19	16.5	7.0	
20	48-67		8.5	6.5-15	

The blood-pressure remained at the last-mentioned level for ten minutes. The experiment was then discontinued.

Under ordinary ether anaesthesia or light chloroform anaesthesia an increase in the frequency of the respirations, a fall in the number of the heart beats and a rise in the blood-pressure occur as in non-anaesthetised animals but the first two effects at least are much less marked than in conscious animals. The largest rise of blood-pressure I have obtained in an anaesthetised rabbit was after a slow injection of 0.4 mg. when the blood-pressure rose from 66 mm. Hg. before the injection to 100 mm. Hg. just after the completion of the injection.

During deep chloroform anaesthesia these results of medullary stimulation are slight and often absent. In this condition the usual and most obvious effect with an efficient dose is marked slowing with increase of depth of the respirations, and if the dose is sufficiently large there may be some irregularity due to an inspiration occurring before expiration is completed. This effect is relatively late in appearing and is frequently accompanied by slight movement in one or more limbs. It is also induced by strychnine and is largely if not wholly due to the conditions associated with increased muscular tone. The effect is shown in figure 1. The animal, a large rabbit, was anaesthetised with chloroform, the blood-pressure was taken from the common carotid artery, and the injections were made into the left facial vein. The respirations were recorded by a modified Gad's method. An injection of 0.2 mg. strychnine hydrochloride was given at 11.15 with little result, and an injection of the same amount of coriamyrtin at 11.36 with little effect until 11.54 when the number of respirations fell rapidly to about half their previous frequency. At 11.55 slight movement of the right hind foot was noted. The slow respiration continued about five minutes but towards the end of this time the respirations increased somewhat in frequency. Afterwards they assumed their previous rapidity notwithstanding the occurrence of slight movements of the foot. The effects of the two subsequent injections are shown in the figure.

As will be seen the effect of the two substances is very similar. Both cause diminished frequency of the respirations, commencing four minutes after the injection and becoming more pronounced a minute later. The effect is most marked with coriamyrtin and this probably accounts for the greater irregularity seen with this drug. The form of the irregularity, it seems to me, points to a peripheral origin for this slowing of the respiration; and it would seem to be due to the increased tone in the respiratory muscles tending to prolong the respiratory phase, associated probably with a diminution in their reactivity to stimuli from respiratory centers depressed by chloroform.

The effect, as has been stated, does not occur in the preconvulsive stage of the normal, or in the decerebrate animal (see fig. 2). In these animals a marked increase in the frequency of the respirations invariably occurs. The explanation is probably to be found in the fact that in the latter case the respiratory center is so power-

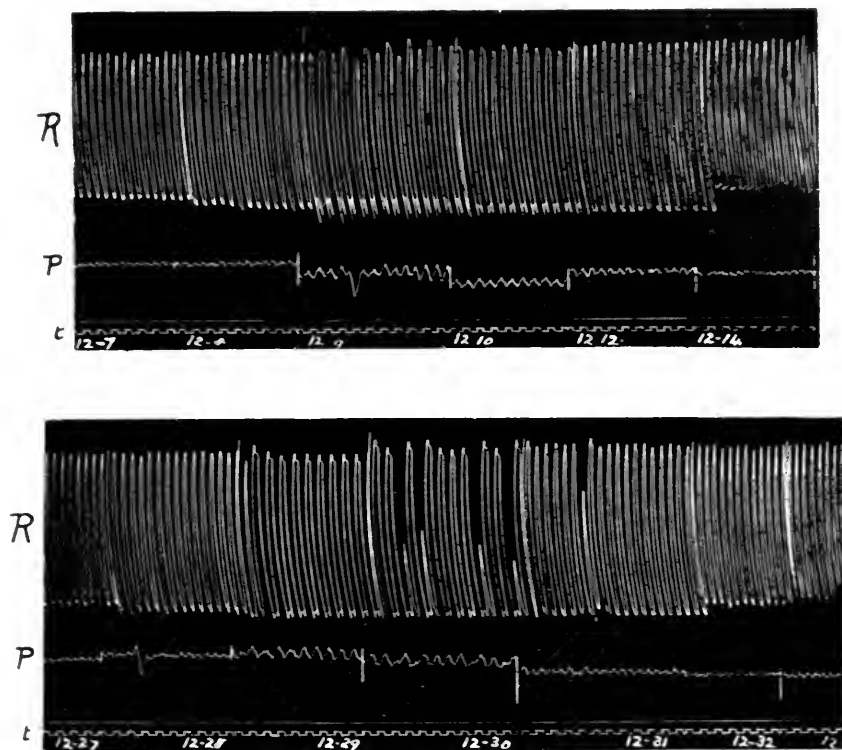


FIG. 1. COMPARISON OF EFFECT OF STRYCHNINE AND CORIAMYRTIN ON THE RESPIRATION OF A DEEPLY ANAESTHETIZED ANIMAL

Rabbit, 2300 grams. Chloroform. 12.04-20—0.2 mg. strychnine intravenously. 12.24—0.2 mg. coriamyrtin intravenously. *R*, respiration (modified Gad's method); downstroke = inspiration; *P*, carotid blood-pressure, base line raised 42 mm.; *t* = time in seconds.

fully stimulated that it overcomes the antagonism of the centers inducing the increased muscular tone, whereas in deeply anaesthetised animals the respiratory center, depressed by the anaes-

thetic, is stimulated to a less extent and the nervous mechanism associated with increase in tone of the muscles becomes dominant. To a certain extent this may be said to occur in normal animals since the convulsions induced by a large dose completely overpower the previous increased excitability of the respiratory center. The fact that the slowing of the respiration in anaesthetised animals may pass away before the limb movements cease must be associated with the more transient action of coriamyrtin on the respiratory center which occurs after large doses under all conditions.

The influence of coriamyrtin on the body temperature was not systematically investigated as this had already been done by Zutz.<sup>12</sup> So far as my observations go they corroborate his, that a fall of temperature occurs in warm-blooded animals after the administration of coriamyrtin. In the case of a rabbit which had received 0.5 mg. per kilogram the temperature fell from 39.5°C. before to 38°C. an hour after the administration.

The antipyretic action of this class of convulsants has been studied by Harnack and his pupils<sup>13</sup> and by Hayashi<sup>14</sup> and others.

#### EFFECT ON THE SPINAL CORD

The convulsions are mainly cerebral in origin. They disappear in the frog when the brain is pithed and do not occur if the brain is pithed just previous to the administration; and in mammals they are more easily elicited when the brain is intact than when the cerebral hemispheres have been removed. It was generally believed that the action of this class of convulsants was solely upon the brain until Gottlieb<sup>15</sup> showed, in the case of picrotoxin, that an increased excitability of the spinal cord was also present. Recently, Fitchett and Malcolm<sup>16</sup> have shown, and I have corroborated their observation, that a similar increased

<sup>12</sup> Loc. cit.

<sup>13</sup> Arch. f. exp. Path. u. Pharm., xlv, p. 272, 1901; xxxviii, p. 397, 1897; xl, p. 151, 1897.

<sup>14</sup> Ibid., l, p. 247, 1903.

<sup>15</sup> Arch. f. exp. Path. u. Pharm., xxx, p. 21, 1892.

<sup>16</sup> Quart. Journ. exp. Physiol., ii, p. 335, 1909.

excitability of the cord occurs in cats after the administration of tutin, a glucoside present in the New Zealand varieties of *Coriaria*. The same increased excitability may be demonstrated with coriamyrtin. For this purpose it is best to use a decerebrate cat. I have shown, in the case of tutin, that the convulsions occurring after the removal of the cerebral hemispheres are easily controlled by even small concentrations of an anaesthetic and that in non-anaesthetised decerebrate rabbits convulsions are not caused by this substance below a section of the spinal cord. The experiment was as follows: A cat, weighing 3400 grams, was anaesthetised with chloroform and the anaesthesia continued with ether. At 3.10 the brain above the quadrate bodies was removed and at 3.25 the spinal cord was cut, with as little disturbance to the blood supply as possible, on a level with the first lumbar vertebra. The anaesthetic was then stopped. Cannulae were introduced into the common carotid artery and the external jugular vein for the purposes of recording the blood-pressure and injecting the drug respectively. The movements of the right fore limb and the left hind limb were recorded by connecting the limbs by means of cords working over pulleys to levers writing on the drums. At 4.8½, forty-three minutes after the anaesthetic had been stopped, 0.5 mg. coriamyrtin was injected intravenously. Powerful tonic-clonic contractions of the fore limbs occurred at 4.9–10 and a minute and a half later well marked but less powerful contractions of the hind limbs. These latter contractions were at first mainly tonic with superadded slight clonus. Later they became more typically clonic in type. At 4.23 the convulsions practically ceased and at 4.24½ a second injection of 0.5 mg. coriamyrtin was given. The result is shown in the tracing (fig. 2).

There is a rise in blood-pressure, a slowing of the heart, and an increase in the frequency of the respiration which, considering the dose, are not very marked, and, almost concurrently, the appearance of convulsions in the hind limbs. (The contractions in the fore limbs were not recorded at this time, but the movements of these limbs were more powerful and of a more clonic type than those of the hind limbs). The early movements of the hind limbs,

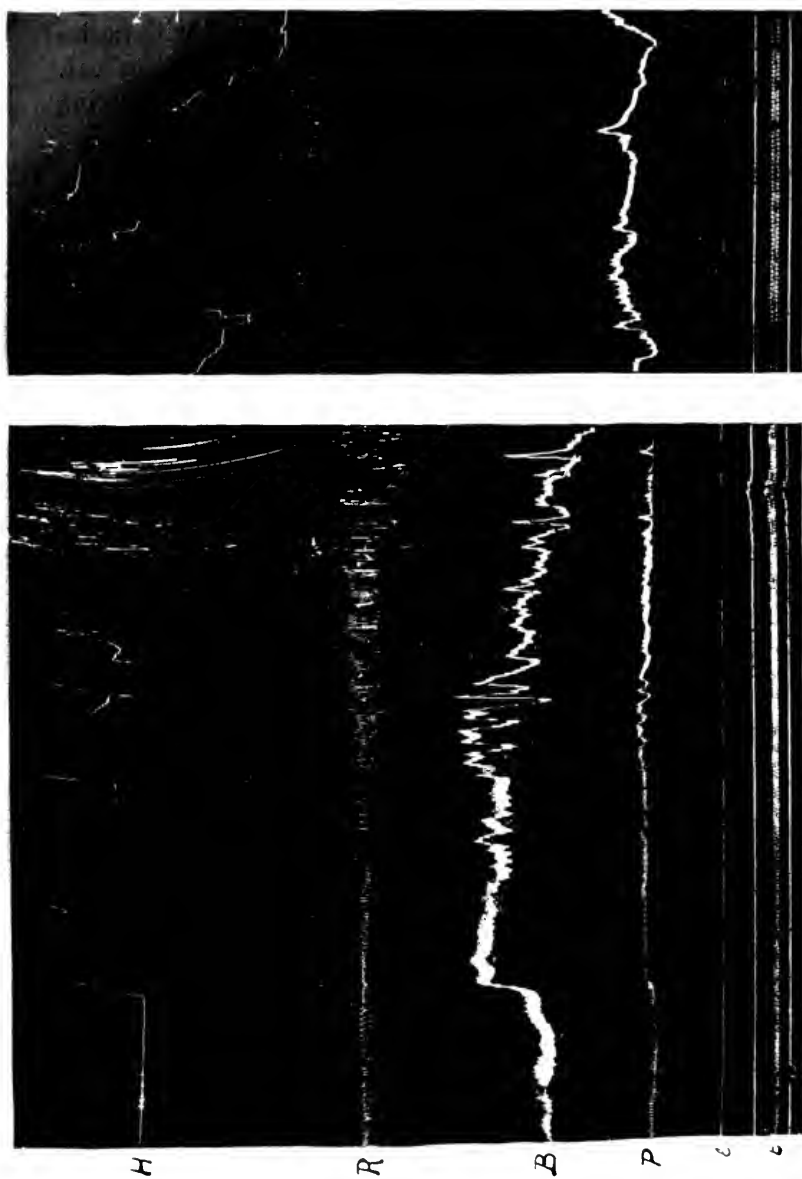


FIG. 2. EFFECT OF CORIAMYRTIN ON HIND LIMB OF DECEBERATE CAT WITH SPINAL CORD DIVIDED  
*H*, movements of hind limb; *B*, blood-pressure with Hürthle's Feder-manometer; *R*, respiration; *t*, base line; *t*, time in seconds; depression on line below = intravenous injection of 0.5 mg. coriamyrtin; interval between tracings = 2½ minutes.

as is shown in the tracing, the upper portion of which is unfortunately incomplete owing to the powerful contractions having carried the writing stilet off the paper, were mainly tonic, but later clonic convulsive movements developed. Still later the contractions again assumed a more tonic type and maintained this form to the end of the experiment.

#### COMPARISON WITH TUTIN

In their paper on the "Physiological Action of Tutin" Fitchett and Malcolm<sup>15</sup> mention experiments on two cats made with the object of comparing the action of tutin and coriamyrtin. Each cat received 3 mg. per kilogram of the drug. The coriamyrtin cat died in forty-two minutes, the tutin cat in thirty-one minutes. This is not in accord with my own observations on rabbits and frogs. In these animals convulsions are produced more quickly and with smaller doses of coriamyrtin than of tutin. The following table of experiments on the same batch of frogs illustrates this point. The numbers indicate the time in minutes between the injection and the first convulsion.

DOSE	CORIAMYRTIN	TUTIN
0.033 mg. per gram body weight.....	3	38
0.066 mg. per gram body weight.....	2	26

In the case of a rabbit to which 0.375 mg. coriamyrtin per kilogram was administered hypodermically, dyspnoea appeared in four minutes and a tonic-clonic convulsion occurred at the end of nine minutes after the injection. A fortnight later 1 mg. tutin per kilogram body weight was injected hypodermically. Dyspnoea appeared in fourteen minutes and no convulsion occurred in the first hour following the injection. Sixty-four minutes after the injection a second similar injection was given. Convulsions commenced one and a half minutes later. These convulsions may have been a delayed effect of the first dose but as I have

<sup>17</sup> Trans. Roy. Soc. Edin., xlvii, pt. ii, p. 315, 1910.

<sup>18</sup> Loc. cit., p. 359.

frequently observed a rapid action after a second dose of tutin it was more probably of the nature of a cumulative action. Usually after a minimal convulsive dose (1 mg. per kilogram) of tutin, convulsions commence about fifty minutes after the administration and after double this dose in about forty minutes after the administration.

The same relative slowness of tutin as compared with coriamyrtin in causing demonstrable effects has invariably been observed in the experiments I have made on anaesthetised animals and also in the one case in which the two substances were administered intravenously to a small normal rabbit. In this case tutin (1.5 mg. per kilogram) was first injected. Dyspnoea commenced in four minutes and the first convulsive symptom appeared in twenty-five minutes after the administration. Five days later 0.25 mg. coriamyrtin per kilogram body weight was given intravenously. Dyspnoea appeared in one minute and convulsions in seven minutes after the injection.

#### SUMMARY

1. The coriamyrtin of Merck appears to be somewhat different from and is probably more powerful pharmacologically than that isolated by Riban.
2. The minimum lethal dose for rabbits is 1 mg. per kilogram body weight.
3. It is quickly eliminated from or destroyed in the body of the rabbit and appears to give rise to no cumulative effects.
4. After minimal doses it causes symptoms of stimulation of the respiratory, vaso-motor and cardio-inhibitory centers in the medulla. These symptoms, however, are not observed, or to only a slight extent, in deeply anaesthetised animals.
5. After large doses it induces convulsions having their origin mainly in the brain, but the excitability of the spinal cord is also increased.
6. Coriamyrtin acts more quickly than tutin.



# ON THE DESTRUCTION OF EPINEPHRIN AND CON- STRICTOR SUBSTANCES OF SERUM BY OXYGENA- TION IN THE PRESENCE OF BLOOD VESSEL WALLS

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An objective proof of the destruction of epinephrin in blood vessel walls has never been made. That it is destroyed in defibrinated blood by oxygenation was first shown by Embden and von Fürth.<sup>1</sup> Yet as early as 1895, Oliver and Schäfer,<sup>2</sup> showed that epinephrin was not destroyed by arterial blood. The remarkably transient character of physiological responses to intravenous injections of epinephrin indicates a very rapid destruction of, or tolerance to, this substance. Carnot and Josserand,<sup>3</sup> Elliot,<sup>4</sup> and Falta and Priestly<sup>5</sup> have given evidences of a depletion of epinephrin in blood after injection into the arterial supply of various organs. The degree of this destruction was found to vary with different organs. To the knowledge of the writer, the site of this destruction has not been determined. It might occur either immediately in the vessel wall itself, or more remotely, in the surrounding tissues.

Towards the solution of this question the following plan suggested itself. Known amounts of epinephrin (Parke, Davis and Company's adrenalin chloride  $\frac{1}{1000}$  solution) were added to each of a series of tubes containing 9 cc. Locke's solution and 1 cc. of serum. These tubes were maintained at approximately

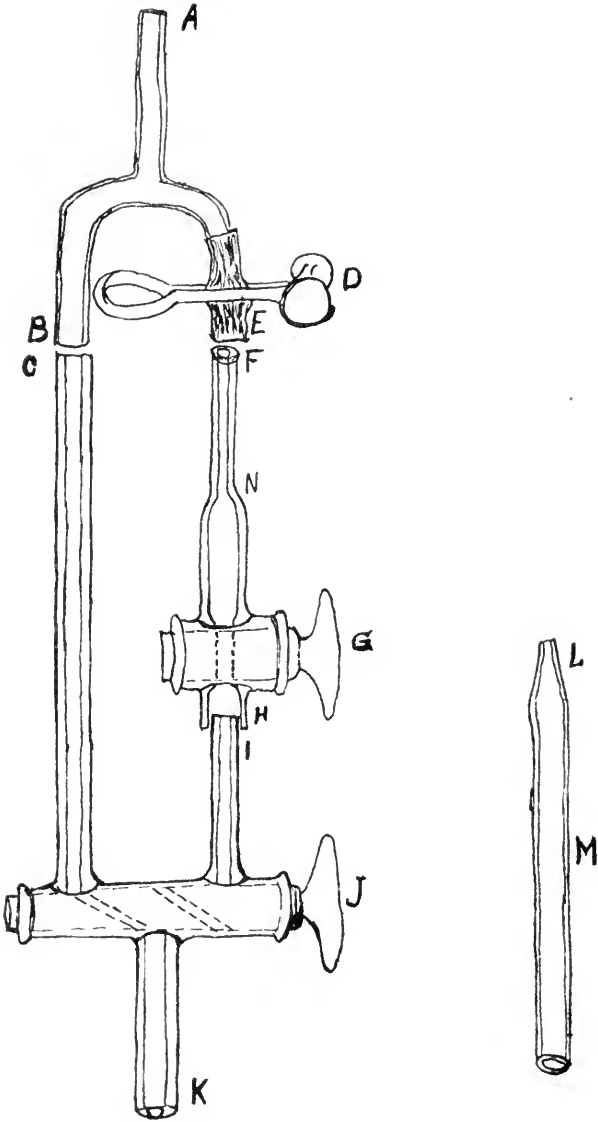
<sup>1</sup> Hofmeister's Beiträge zur Physiol. u. Path., iv, 421, 1904.

<sup>2</sup> Journ. of Physiol. xviii, 230, 1895.

<sup>3</sup> Comptes Rendus de la Societe de Biologie, liv, 1472, 1902.

<sup>4</sup> Journ. of Physiol. xxxii, 427, 1905.

<sup>5</sup> Berliner klinische Wochenschrift, xlviii, 2102, 1911.



37°C., and arranged for oxygenation. Into one tube was placed from one to three grams of absolutely fresh artery wall from the same animal that furnished the serum. The artery wall material in most cases was from the abdominal aorta and its larger branches. This was split open, carefully washed in warm Locke's solution, then quickly placed into the epinephrin-serum mixture. Oxygen was led through to the bottom of the solutions to be oxygenated, by drawn glass tubes, in order to force the gas through the solutions in a constant and approximately equal stream of small bubbles. The presence or absence of epinephrin was determined by the frog perfusion method of Loewen<sup>6</sup>-Trendelenburg.<sup>7</sup>

The original technique of this method has been frequently described. Ringer's solution is conducted from a Mariotte flask through a rubber tube to a cannula placed in the abdominal aorta of a large frog. Vasomotor changes are measured by changes in rate of out-flow from a cannula placed in the anterior abdominal vein. The height of the Mariotte flask is so adjusted as to obtain any desirable rate. The fluid from the vein cannula is allowed to drop onto an electro-magnetic drop recorder, marking on a revolving drum. The usual method of procedure is to inject the solution to be treated into the rubber tube close above the aortic cannula. An injection of 1 or 2 cc. will temporarily raise the head of pressure. To obviate this, I have devised a simple modification which has proven perfectly satisfactory in this laboratory. A two-way system of tubes is used, terminated at the lower end by a three-way stop cock, *J*, with 2 mm. bore tubes. The single tube, *K*, is connected to the aortic cannula. The paired tubes are connected above by a small T-tube; *A*, the single tube of which is connected to the Mariotte's flask. One arm of the paired tubes is cut short and replaced by a removable section, *F*, *N*, *H*. This removable section is prepared from a two-way glass cock and tubes. The lower arm, *H*, is cut off about 0.5 inch from the cock and fitted to the short arm, *I*, of the paired tubes of the three-way cock. By means of a soft rubber tube doubled back onto itself, covering both the inside and outside of the short stub, *H*, of the two-way cock, an easy air-tight connection can be made. The longer arm of the two-

<sup>6</sup> Loewen, Arch. Exp. Path. u. Pharm. li, 415, 1904.

<sup>7</sup> Trendelenburg, P. Arch. Exp. Path. u. Pharm. lxiii, 161, 1910.

way cock is drawn out to a small bored tube at such a distance, *N* from the cock that when this arm is about the same length as the long arm of the three-way cock, it will hold approximately 1 cc. of solution. By means of a soft rubber tube, *E*, this removable section is connected at the upper end of the two-way system to the T-tube from the pressure bottle. The detachable section is conveniently filled by inserting the pointed end, *L*, of a small glass tube, *M*, into *H*, drawing the test solution, pipette fashion, up *F* to fill the whole tube, also including the lower end of *L*. Then the glass cock, *G*, is closed, and the tube, *M*, removed, the removable section placed in connection at *E* and *I*. The pinch cock *D*, on the rubber tube *E*, is released, *G*, now opened, and the two-way-cock, *J*, is turned so that the circuit is opened through the detachable section, *A*, *N*, *I*, *K*. Thus the test solution is forced into the vascular system of the frog under the same head of pressure as forced the Locke's solution for the normal perfusion via circuit *A*, *B*, *C*, *K*. The only interruption to the flow between *A* and *K* is during the time of turning of the cock *J*.

The following experiment, presented in tabular form, is typical of the eight experiments performed:

TABLE 1

*This table shows the preparation of each solution perfused*

NUMBER OF SOLUTION	EPINEPHRIN ADDED*	SERUM	LOCKE'S SOLUTION	ARTERY	VEIN	DURATION OF OXYGENATION
	cc.	cc.	cc.	grams	grams	hours
1	0	1	9	0	0	0
2	0	1	9	0	0	1
3	1	1	8	0	0	0
4	1	1	8	0	0	1
5	1	1	8	2.9	0	0
6	1	1	8	2.9	0	1
7	1	1	8	0	2.8	1

\* "Adrenalin chloride"  $\frac{1}{100000}$ .

Each of the above solutions was diluted to five volumes with Locke's solution before injection.

TABLE 2

*This table shows the results of perfusion of 1.3 cc. of the above diluted solutions. A new injection is not made until the rate of outflow following an injection has closely approximated the rate previous to injection. A frog weighing about 50 grams had been perfused continuously with Locke's solution for six hours before beginning this series.*

NUMBER OF SOLUTION	DROPS PER MINUTE BEFORE INJECTION	DROPS PER MINUTE DURING HEIGHT OF ACTION	CHANGE IN RATE	PERCENTAGE DECREASE IN RATE OF OUTFLOW	PERCENTAGE LOSS IN CONSTRICTING ACTIVITY*
1	15.0	7.2	7.8	52	Standard
2	13.0	6.6	6.4	49	5.7
3	11.0	5.5	5.5	55	Standard
4	13.2	5.8	7.4	56	0
5	10.0	6.0	4.0	40	27.0
6	11.0	11.0	0.0	0	100.0
7	11.6	7.2	4.4	37	32.0

\*In order to calculate the percentage loss of constrictor substances, it seemed wise to divide the series into two groups: In the first are the two solutions without the epinephrin, no. 1 and no. 2, and in the second, those solutions with epinephrin, nos. 3, 4, 5, 6, and 7. The control solution of each series then serves as a basis for comparison.

As can be seen from table 2, all constrictor effect of the serum-epinephrin mixture disappeared when oxygenated in the presence of artery wall, as in solution no. 6. Solution no. 5, with artery wall but not oxygenated, and solution no. 7 with vein wall and oxygenation, each have about 30 per cent of the constriction action of the control solution, no. 3.

It is well known that serum contains one or more constrictor substances, which act in many respects similarly to epinephrin solutions when perfused through the peripheral vessels of the frog.<sup>8</sup> Furthermore, while Oliver and Schäfer (l. c.) showed that defibrinated blood does not destroy epinephrin, Trendelenburg (l. c.) showed that serum would protect it from oxidation. This last fact we have been able to confirm repeatedly, and can extend the observation to include defibrinated blood, hirudin-blood, and hirudin-plasma. A solution of egg albumin, we found, was not protective. Yet, a serum in contact with artery

<sup>8</sup> O'Connor, Münchener medizinische Wochenschrift, lviii, 1439, 1911.

wall loses not only its power to protect epinephrin against oxygenation, but the serum itself undergoes some change whereby it no longer produces constriction.

The experiment illustrated above, using serum, aorta and vena cava in epinephrin solutions, was confirmed in two independent experiments with dogs, two with goats, and four with rabbits. The results were striking in every case. The results so far obtained show that epinephrin, together with those pressor substances that are found in serum and are measured by the constriction produced on perfusion through the peripheral vessels of the frog, rapidly disappear when oxygenated in the presence of artery wall.

## ACTION OF QUININE ON THE LEUCOCYTES

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More than forty years ago in a very exhaustive research on quinine Binz (1) observed a diminution in the number of leucocytes in the peripheral circulation following the administration of this drug. He found that by giving large doses of the hydrochloride to young cats the leucocyte count would drop after several hours from a normal of 171 to 26 in twenty fields, a reduction to about one-seventh of the usual number.

Many years later Wilkinson (2) partially confirmed these findings on rabbits. However, a leucopenia could be produced only by giving toxic doses of the drug while if smaller doses were used a leucocytosis would result. No mention was made by him of the kind of cells affected during the leucopenic period but when the leucocytosis occurred after the administration of small doses he found a relative increase in the mononuclear variety.

Maurel (3) also investigated its action upon rabbits and obtained a marked reduction in the number of leucocytes.

Quite recently Askenstedt (4) made some observations on the blood changes produced by quinine in man and found that when quinine sulphate was given for a period of days there occurred a rapid reduction of haemoglobin and red blood cells while the white blood cells were increased in number. Only two differential counts of the white blood cells were made, both of which showed a decrease in the mononuclear elements and an increase in the polynuclear forms. Inasmuch as the work of Binz (1) appeared before the more modern methods of blood examination were in vogue and since no complete study was

later made of the kind of cells affected by the drug, further investigation may well be made along these lines.

The present work was begun on man but more extensive experiments were performed on the dog. The cat was also used, and confirmed the findings in the dog.

A satisfactory classification of the leucocytes of blood is difficult to make, but for the sake of simplicity their usual separation into six types was adopted, namely: (1) polynuclears, (2) lymphocytes, (3) transitional and large mononuclears, (4) mast cells, (5) eosinophiles, (6) degenerates and other forms. An effort was made to separate the small lymphocytes from the large variety, but the results were so variable that it was thought advisable to group both forms under the head of lymphocytes.

No detailed description need be given of the forms found, as reference may be made to the work of Dawson (5) and Busch and Van Bergen (6) upon the dog's leucocytes, Hirschfeld (7) for the blood of the cat, and Schleip (8) and Weidenreich (9) for the blood of man.

Food was withheld from the animals from twenty-four to forty-eight hours prior to the experiments, with the exception of those animals that were used for obtaining the splenic tracings.

In the animals from which splenic tracings were obtained the blood for examination was taken from the femoral artery by puncturing it with an ordinary needle. In all other animals the blood was taken from the nose, the puncture being made by the blood stickler ordinarily used in clinical work. The blood was withdrawn from the finger in the usual way when man was the subject.

As a preliminary in the cat, the nose was first shaved, then washed with soap and water, rinsed with alcohol and allowed to dry. In the dog the shaving was omitted. In this way no trouble was encountered in obtaining the blood as was experienced in the beginning, when the ear was used. Excitement causes the dog's nose to sweat and often a drop will not collect. To avoid this the skin should be wiped dry before obtaining the sample for examination.



Food was not withheld from man but the ordinary leucocytosis of digestion was studied by observing the hourly changes in the number and kinds of leucocytes, and the drug was administered on a subsequent day. A comparison could then be made and the factor of the leucocytosis of digestion could be easily eliminated.

The Thoma-Zeiss haemocytometer was used to estimate the number of white blood cells, the diluting fluid being a 0.5 per cent acetic acid. For the differential examination 500 cells were counted. Wright's (10) method of staining the leucocytes was used throughout and at least two spreads were used in making the count.

The salts of quinine used were the hydrochloride and the sulphate, both of which were obtained from Merck and Company.

#### ADMINISTRATION TO DOGS BY MOUTH

Quinine hydrochloride was given in solution to dogs by means of a stomach tube in doses of 0.050 gram to 0.100 gram per kilogram of body weight. If the smaller dose was given vomiting did not occur, but when the larger was administered emesis occurred about a quarter of an hour after and continued at intervals for about an hour. By this procedure a reduction in the total number of leucocytes was obtained of from 2500 to 3000 in the course of an hour. The count remained low for several hours when an increase of 2000 to 2500 occurred. The differential count showed at the end of an hour an absolute reduction of both lymphocytes and polynuclears with a 10 to 15 per cent greater decrease in the former. There was also a relative decrease in lymphocytes while the relative per cent of polynuclears would remain unchanged or would be slightly increased. When the increase in the total number of cells occurred after several hours the polynuclears were mainly affected, the relative and absolute per cents of polynuclears were increased while the lymphocytes always showed a relative decrease.

## ADMINISTRATION TO DOGS SUBCUTANEOUSLY

Quinine hydrochloride was given to dogs subcutaneously in doses of 0.050 gram to 0.150 gram per kilogram of body weight. In these animals vomiting always occurred about half an hour after the administration of the drug. A reduction of from 1000 to 4000 leucocytes appeared within an hour. There was, however, a preliminary increase which occurred about half an hour after the quinine was given. Following the reduction there was an increase several hours later.

When the preliminary increase occurred the differential count showed a slight relative and a marked absolute increase in lymphocytes with a slight relative and absolute decrease in polynuclears. In the second or leucopenic stage we have substantially the same differential findings as when the drug was given by stomach, namely, an absolute reduction of both lymphocytes and polynuclears, the decrease being mainly with the lymphocytes. Similarly when the second increase occurred the polynuclears were affected almost entirely.

## PROLONGED ADMINISTRATION TO THE DOG

In order to ascertain whether marked changes could be produced by repeated administration of quinine, a dog was given daily doses of the drug by mouth for a period of two weeks. At the beginning of the period amounts from 0.050 gram to 0.100 gram per kilogram were given daily without producing definite blood changes. The dose was then increased to 0.300 gram and later to 0.400 gram per kilogram. The dose was then reduced to 0.150 gram per kilogram and repeated until the death of the animal which occurred fifteen days after the experiment was begun. The following table will show the effects, the striking features being a marked reduction in lymphocytes and eosinophiles and an increase in polynuclears. The autopsy showed the spleen to be atrophic and the stomach congested.

*Experiment A—Table showing the effect of repeated administrations of quinine to a dog*

DATE AND HOUR	WHITE BLOOD CELLS	POLYNUCLEARS	LYMPHOCYTES	TRANSITIONAL AND MONONUCLEARS	EOSINOPHILES	MASTS AND OTHER FORMS	REMARKS
1912		per cent	per cent	per cent	per cent	per cent	
1-10, 2.00 p.m.	10,695	66.0	17.0	7.4	7.0	2.6	0.050 g. per kilo. quinine sulph. in capsules.
1-11, 2.00 p.m.	11,204	67.0	14.4	6.6	10.4	1.6	0.050 g.
1-12, 2.00 p.m.	16,297	68.2	17.6	4.8	8.6	0.8	0.050 g.
1-13, 2.00 p.m.	13,496	64.8	15.0	6.0	13.0	1.2	0.050 g.
1-14, 2.00 p.m.	11,204	65.0	18.0	6.0	10.0	1.0	0.050 g.
1-15, 2.00 p.m.	15,278	56.6	20.6	7.0	15.0	0.8	0.100 g.
1-16, 2.00 p.m.	10,441	60.2	19.2	8.0	12.0	0.6	0.100 g.
1-17, 2.00 p.m.	8,403	61.2	20.0	7.0	10.2	1.6	0.150 g.
1-18, 2.00 p.m.	8,912	57.2	19.0	11.4	12.0	0.4	0.150 g.
1-19, 2.00 p.m.	11,714	49.4	22.8	13.6	14.0	0.2	0.200 g.
1-20, 2.00 p.m.	8,403	51.3	23.5	9.0	13.2	3.0	0.200 g.
1-21				no-count			0.200 g.
1-22, 8.30 a.m.	7,130	61.0	21.8	8.4	8.4	0.4	0.200 g. at 8.30 a.m. also at 4.30 p.m.
1-23, 8.30 a.m.	8,403	78.6	9.4	9.6	1.8	0.6	0.200 g.
1-24, 8.30 a.m.	8,912	76.6	8.0	12.2	2.6	0.6	0.150 g. at 8.30 a.m. 0.150 g. at 4.30 p.m.
1-25, 8.30 a.m.	9,421	79.4	7.4	12.2	0.2	0.8	0.150 g. at 8.30 a.m. 0.100 g. at 4.00 p.m.
1-26, 8.30 a.m.	16,552	84.4	3.2	9.8	1.2	1.4	0.150 g., vomited.

Animal was in partial stupor since 9.30 a.m. Died during the night.

#### ADMINISTRATION TO THE CAT SUBCUTANEOUSLY

The work with the cat was limited to but one experiment as the results ran parallel with those of the dog. From the subcutaneous injection of 0.200 gram of quinine sulphate per kilogram made soluble by forming the bisulphate with a few drops of dilute sulphuric acid, a marked reduction in number was obtained. The findings were identical with those of the dog, except that

they were accentuated throughout and so no detailed account need be given. The death of the animal occurred the next day.

#### ADMINISTRATION TO MAN BY MOUTH

When man was the subject of the experiment the findings were not so clear cut as they were in the animals, but conclusions could be drawn which in most respects resembled the findings in the cat and dog. From the use of 0.6 gram to 1 gram of either the hydrochloride or sulphate in capsules, an actual decrease or an inhibition of the usual leucocytosis of digestion was observed. The most striking effects noticed were in the kinds of leucocytes affected. The usual result was a slight increase in the lymphocyte count which lasted for several hours and was then followed by a marked decrease. The polynuclears were little affected at first, whereas later they were increased in number.

In considering the above findings several possibilities suggested themselves as causes operating to produce such results. Since lymphocytes were affected mainly by this drug, the organs where they were formed in the body were looked upon as capable of throwing light upon the question. As quinine was known to influence the splenic movements this organ was studied with the view of determining the rôle it played and of ascertaining its influence in producing these blood changes if any. For this purpose dogs were used. After being anaesthetized with morphine and chloretone,<sup>1</sup> the external jugular vein was dissected free and a cannula inserted into it. The spleen was then isolated, enough of the blood supply ligated to enable one to place it in an oncometer and a graphic record of its movements obtained by means of a piston recorder. By this method it was found that the spleen contracted markedly after the injection into the jugular vein of 0.100 gram of quinine hydrochloride. Blood was taken before and also ten to fifteen minutes after the injection of the quinine solution. A marked reduction in white blood cells was produced but in spite of the loss in numbers there was a slight

<sup>1</sup> Morphine 0.1 gram to 0.2 gram given hypodermically; chloretone 3 grams to 4 grams per stomach.

relative increase in the lymphocytes. To ascertain the effect of the splenic contraction a later experiment was conducted on another animal in which the spleen was stimulated reflexly by stimulation of the central end of the vagus. The following table will show the relative and absolute relation of lymphocytes to polynuclears in such an experiment.

	W. B. C.	LYMPHOCYTES	POLYNUCLEARS
Before splenic contraction.....	15,533	13.6 per cent—2,112	82.0 per cent—12,737
After.....	21,645	17.6 per cent—3,809	76.4 per cent—16,536

This shows an increase in white blood cells, a relative increase in lymphocytes, a relative decrease in polynuclears and an absolute increase in both lymphocytes and polynuclears. We have about a 30 per cent absolute increase in lymphocytes so that the increase after contraction of the spleen is seen to be mainly a lymphocytosis. We are therefore led to think that the initial increase which occurs shortly after the drug is given subcutaneously may be due to mechanical forces entirely, namely, the contraction of the organs which harbor large numbers of lymphocytes. If we compare the following experiment with the one above we see a likeness in the first stage where the initial increase occurs.

*Dog.—Effect of quinine when given at short intervals*

TIME	W. B. C.	LYMPHOCYTES	POLYNUCLEARS	REMARKS
9.15 a.m.	23,937	15.8 per cent—3,782	78.8 per cent—18,862	
9.16 a.m.				0.050 g. per kilo quinine hydrochloride subcut.
9.40 a.m.	24,446	20.0 per cent—4,889	72.6 per cent—17,747	
10.15 a.m.	19,868	13.6 per cent—2,702	75.6 per cent—15,020	
10.30 a.m.				0.025 g. per kilo
11.15 a.m.	20,626	10.8 per cent—2,227	81.6 per cent—16,830	
11.30 a.m.				0.050 g. per kilo
1.15 p.m.	31,800	6.6 per cent—2,098	84.2 per cent—26,775	

Relaxation follows a few minutes after the contraction caused by the quinine and the organ gradually returns to a normal condition. Bruce (11) found that the leucopenia produced by peptone was due to the leucocytes being harbored mainly in the spleen, lungs and liver. Binz (1) found that equal quantities of a 1 to 2000 solution of quinine hydrochloride and pure blood lessened or stopped the movements of the white blood cells and from the very large doses which I found it was necessary to give in order to produce definite changes in the differential count quinine may very well exist in the blood in such a concentration which would seriously affect the cells and so explain the leucopenia.

If we consider the cause of the leucopenia to be the destruction of the leucocytes then Löwits' (12) explanation of the leucocytosis following a leucopenia may be used, namely that the increase is due to a chemical stimulation of the blood forming organs by substances thrown into the blood by the disintegration of the leucocytes. This is not improbable for the degree of leucocytosis varies almost directly with the degree of leucopenia.

To recapitulate: Quinine, when given to animals in single doses, produces changes in the number of leucocytes which can be divided into three phases. The first, or stage of preliminary leucocytosis, appears shortly after the drug is given and is no doubt caused by the contraction of the spleen and other tissues acted upon by the drug, the increase affecting the lymphocytes for the most part.

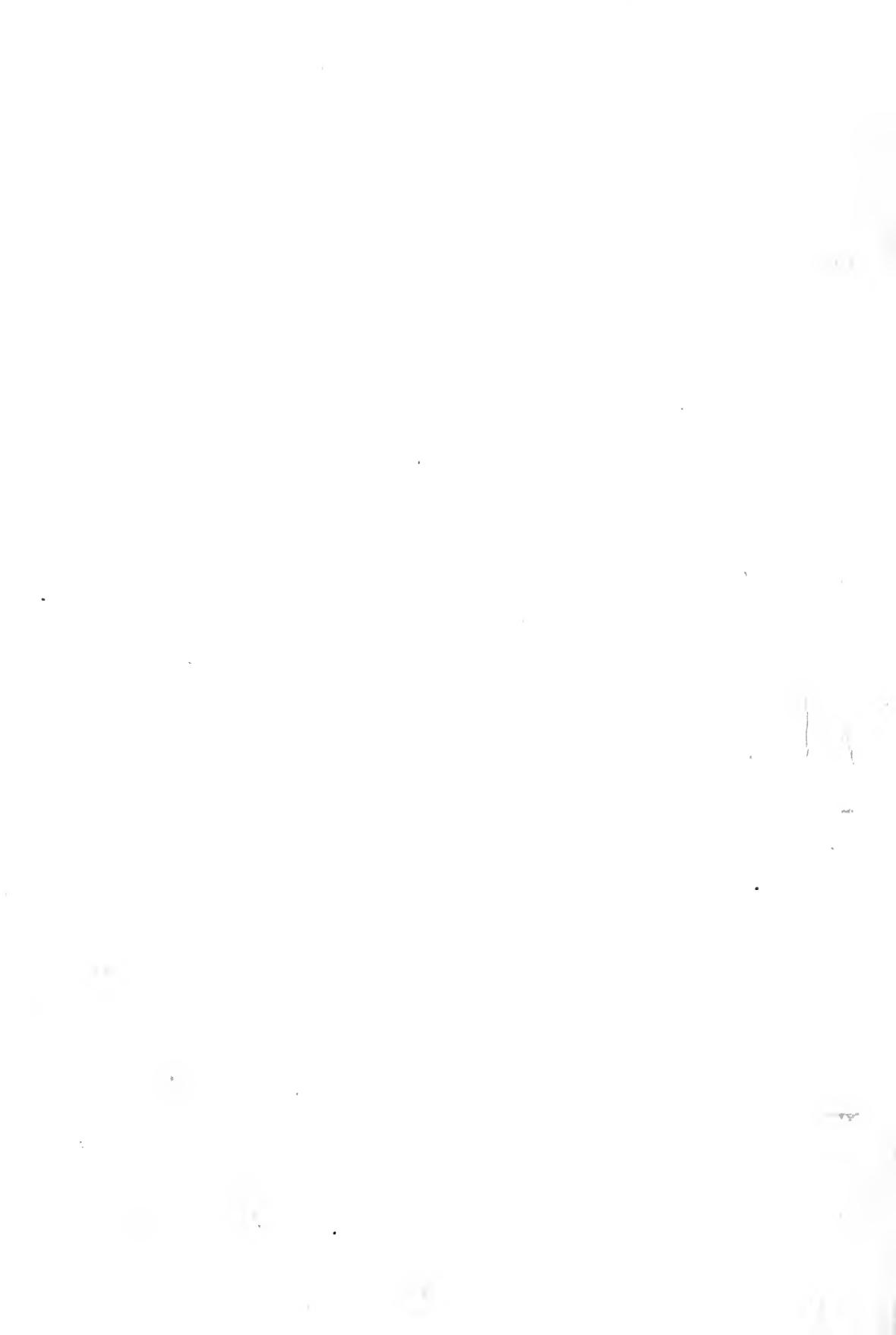
An hour or two later the second or leucopenic phase appears in which there is a reduction of both lymphocytes and polynuclears but mainly of the former.

This phase is subsequently followed by the third stage, a phase of secondary leucocytosis which is quite marked, the polynuclears alone being increased, while the lymphocytes remain low or continue to decrease in number.

When the drug is administered to the dog continuously for weeks death results and the blood picture is essentially the same, namely a leucopenia followed by a leucocytosis, the period of leucopenia showing a great decrease in lymphocytes and eosinophiles while the period of leucocytosis affects the polynuclears.

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# THE ANAPHYLACTIC REACTION OF PLAIN MUSCLE IN THE GUINEA-PIG

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## I. INTRODUCTORY

It has been made clear by the work of Auer and Lewis,<sup>1</sup> Biedl and Kraus,<sup>2</sup> Anderson and Schultz,<sup>3</sup> Schultz and Jordan,<sup>4</sup> among others, that the peculiarly and rapidly fatal character of anaphylactic shock in the guinea-pig depends on the valve-like closure of the bronchioles. This would seem to be due partly to the special susceptibility of the bronchiolar musculature in this species, partly to the thick and folded mucous membrane with which the bronchioles are lined, and which effectively blocks the lumen when the muscular coat constricts the tube (Schultz and Jordan). It has been proved that the effect on the bronchiolar muscle is due to a direct peripheral action, unmodified by section of the vagi or even by the degeneration of their fibers (Auer<sup>5</sup>). It is not possible here to give a detailed review of the different theories put forward to explain the anaphylactic condition, or of the evidence by which they have been supported. Nor is it necessary, since several comprehensive reviews of the literature are already available (Richet,<sup>6</sup> Friedemann,<sup>7</sup> Schitten-

<sup>1</sup> Journ. Amer. Med. Assoc., liii, p. 458, 1909; Journ. of Exper. Med., xii, p. 151, 1910.

<sup>2</sup> Wien. klin. Wochenschr., xxiii, p. 385, 1910.

<sup>3</sup> Proc. Soc. Exp. Biol. and Med., vii, p. 32, 1910.

<sup>4</sup> Journ. Pharm. and Exp. Therap., ii, p. 375, 1911.

<sup>5</sup> Journ. of Exp. Med., xii, p. 638, 1910.

<sup>6</sup> L'Anaphylaxie, Paris, 1912.

<sup>7</sup> Jahresb. über d. Ergeb. d. Immunit., vi, Abt. i, p. 31, 1911.

helm,<sup>8</sup> Doerr<sup>9</sup>). It will suffice to mention the main tendencies of speculation on the subject. We may note, on the one hand, the adaptation of the theory of "sessile receptors" originally put forward to explain the distinct, but in some respects analogous phenomenon of supersensitiveness to the true toxins. This conception, as put forward by Besredka<sup>10</sup> and by Friedberger,<sup>11</sup> has been recently somewhat overshadowed in significance by the theory attributing the poisonous action of the reinjected antigen to the liberation, through the action of a specific ferment, of a non-specific poisonous product of partial protein hydrolysis. It has been shown (Biedl and Kraus,<sup>12</sup> Vaughan,<sup>13</sup> Schittenhelm and Weichardt<sup>14</sup>) that various products of partial protein hydrolysis produce, on injection into the normal animal, symptoms in many respects resembling those of the anaphylactic shock. On the other hand, an increased excretion of the products of protein katabolism has been shown to follow the reinjection of an antigen into an animal prepared by previous injections (Friedemann and Isaac, Schittenhelm and Weichardt), and different observers have elicited symptoms resembling those of the anaphylactic shock by injecting, into a normal animal, serum from a sensitised animal digested with the antigen (Richet,<sup>15</sup> Vaughan,<sup>16</sup> Anderson and Frost<sup>17</sup>), or serum from a normal animal, digested with specific precipitates (Friedberger<sup>18</sup>), or with blood corpuscles sensitised with haemolysin (Friedemann<sup>19</sup>). There are many variants of this "parenteral digestion" theory: thus the specific antibody concerned in bringing the antigen into relation with the digesting complement is identified with precipitin (Fried-

<sup>8</sup> *Ibid.*, p. 115.

<sup>9</sup> *Zeitschr. f. Immunit. (Ref.)*, p. 49, 1910.

<sup>10</sup> *Ann. de l'Institut Pasteur*, xxii, p. 496, 1908.

<sup>11</sup> *Zeitschr. f. Immunit.*, ii, p. 208, 1909.

<sup>12</sup> *Wien. klin. Wochenschr.*, xxii, p. 363, 1910.

<sup>13</sup> *Journ. of Infect. Dis.*, iv, p. 476, 1907.

<sup>14</sup> *Munch. med. Wochenschr.*, p. 1098, 1912.

<sup>15</sup> *C. R. Soc. de Biol.*, lxvi, p. 1005, 1909.

<sup>16</sup> *Zeitschr. f. Immunit.*, xi, p. 673, 1911.

<sup>17</sup> *Hygienic Lab. Bulletins*, No. 64, Washington, 1910.

<sup>18</sup> *Zeitschr. f. Immunit.*, iv, p. 636, 1910.

<sup>19</sup> *Ibid.*, ii, p. 591, 1909.

berger,<sup>20</sup> Doerr, and Russ<sup>21</sup>) or regarded as a distinct type of antibody. Again, the proteolytic formation of the non-specific poison is located by some in the circulating fluids, by others in the cells participating in the anaphylactic reaction—a view which unites the conceptions of “sessile receptors” and parenteral digestion. Recently Doerr and Moldovan<sup>22</sup> have called in question the validity of the evidence for the fermentative production of an intermediate poison as the cause of the anaphylactic reaction, and have suggested that an immediate physical change in the blood colloids accounts for the effect. It may be said, then, that there are two main questions at issue between rival theorists on the subject.

1. Does the immediate reaction between antibody and antigen, which results in the anaphylactic shock, take place in the circulating fluids, or in the responsive tissues?

2. Is the shock dependent directly on a physical change, or on the production of a poisonous digestion-product (“Apotoxin” of Richet, “Anaphylatoxin” of Friedberger)?

It would seem that the first question could be settled by isolating, from the circulation of the sensitive animal, a tissue which *in situ* shows a characteristic anaphylactic response. This can be done by transplantation into a normal individual, as in the beautiful experiment of Bloch,<sup>23</sup> in which a skin-graft, from a subject giving a cutaneous reaction to extracts from a certain Tricophyton, retained its specific supersensitiveness after healing on to a normal person, whose skin remained elsewhere normally insensitive. It might be felt, however, that this, though undoubtedly an anaphylactic phenomenon, was of too special a nature to be made the basis of general conclusions.

In the case of the anaphylactic shock as seen in the guinea-pig, of which the dominating feature is contraction of plain muscle, the rival possibilities of direct action on the tissues or primary humoral reaction, can be tested by isolating portions

<sup>20</sup> Zeitschr. f. Immunit., ii, p. 208, 1909.

<sup>21</sup> Ibid., iii, p. 706, 1909.

<sup>22</sup> Biochem. Zeitschr., xli, p. 27, 1912.

<sup>23</sup> Bloch and Massini: Zeitschr. f. Hyg. u. Infect., lxiii, p. 68, 1909.

of plain muscle and freeing them from body fluids. Magnus and others have indicated methods for keeping such isolated plain-muscular tissues in a state of vitality and sensitiveness, and these have been applied by Schultz<sup>24</sup> to the problem under discussion. This observer used chiefly short lengths of small intestine, removed from the etherised animal, and suspended in a warm oxygenated saline bath. A few experiments were made with other plain muscular organs. Schultz concluded that the isolated plain muscle was in itself supersensitive to the protein to which the whole animal had been sensitised by a previous small injection. Unfortunately the evidence, on which this conclusion is based, is not such as to inspire the conviction that the phenomenon can be wholly so explained. I shall have occasion for detailed criticism of some of Schultz's results in later sections. Here it is sufficient to indicate the general nature of his evidence. Schultz found that the isolated plain muscle of the guinea-pig responded by marked tonus to various sera and other foreign proteins. Plain muscle, from a guinea-pig rendered anaphylactic to horse serum, usually responded to this serum with higher and more prolonged tonus, and more marked rhythm, than that evoked by the same dose in a preparation made from a normal animal in as closely similar a manner as possible. Further doses of horse serum, after the first, caused further contractions: these were usually, but not always, smaller than the first. The difficulty is at once experienced of reconciling these observations with the known characteristics of the anaphylactic condition, particularly its sharp contrast with the normal, and its removal (antianaphylaxis) by an effective reinjection. A guinea-pig sensitive to horse serum may be killed in a few minutes by intravenous injection of 0.01 cc. of the serum; a normal guinea-pig will take 5 cc. or more of the same serum by the same route with hardly noticeable symptoms. It is hardly credible that such a contrast depends on a difference in plain-muscle sensitiveness so small as that which Schultz's curves suggest. They suggest, indeed, a difference of sensitiveness of

<sup>24</sup> Journ. Pharm. and Exp. Therap., i, p. 549, 1910, ii, p. 221, 1910; Hygienic Lab. Bulletins No. 80, Washington, 1912.

the same order as that which can be observed between individual specimens of plain-muscle from normal individuals. For example, in a figure facing page 30 of the Hygienic Laboratory Bulletin No. 80, Schultz shows the contraction of a 35 mm. segment of bowel, from a sensitised guinea-pig, in response to 1 cc. of horse serum added to 10 cc. of oxygenated Ringer's solution. The maximum height of the resulting curve is 53 mm. from the initial level, according to my measurement. It contrasts fairly effectively with the reaction of a similar segment from a non-sensitised pig taken at the same time. But in the second of three figures, following page 34 of the same bulletin, he shows the response of a 25 mm. segment of normal guinea-pig's bowel to an equal dose of normal guinea-pig serum. The curve in this case, when allowance is made for the indicated reduction in reproducing the tracing, rises to a maximum 60 mm. from the original level. Now even if the anaphylactic shock were merely a slight and not regularly occurring exaggeration, of the reaction which an animal gives to the injection of all sera, including its own, this could hardly be regarded as a convincing demonstration of its occurrence in the isolated organ. But true anaphylaxis consists in a sensitiveness so extreme, and so highly specific, that one is left in doubt as to whether such difference as is demonstrated by Schultz's experiments is a truly anaphylactic phenomenon at all. Richet has stated that an animal, which has been rendered anaphylactic to any substance, shows, in addition to the specific supersensitiveness to that substance, a tendency to react excessively with poisons in general. It could legitimately be suggested, by an adherent of the humoral theory, that Schultz's results merely indicate such a general increase of excitability in the plain muscle of the sensitised animal. If it were admitted that he has demonstrated a genuine tissue-anaphylaxis of guinea-pig plain muscle, it might well be assumed that this is only a subsidiary factor in the production of shock, the main part of the effect being presumably due to humoral elaboration of a poison (cf. Friedemann<sup>25</sup>); or an effect of such

<sup>25</sup> Jahresb. über d. Ergeb. d. Immunit., vi, Abt. i, p. 94, 1911.

minor degree might even be attributed to interaction between the horse serum added and the small residue of blood in the vessels of the isolated intestinal muscle.

My own experiments began some time ago with the accidental observation of the extraordinary sensitiveness to horse serum of certain isolated uteri, which I was using for another purpose. Enquiry revealed the fact that the guinea-pigs from which they were taken were all survivors from antitoxin standardisations. Testing the uterus from a normal guinea-pig I found no response of the kind. I was proceeding to investigate the matter further when Schultz's preliminary paper appeared. I dropped the investigation temporarily, and resumed it on the publication of Schultz's results in full, since it seemed clear to me that, by attention to certain details, it would be possible to give a more convincing demonstration of tissue sensitisation, and to show, with guinea-pig's plain muscle, freed from all traces of blood, a sensitiveness so exquisite, and so closely specific for the sensitising antigen, that there was no possibility of regarding it as other than a principal factor in the plain-muscle anaphylactic reaction, as seen in the whole guinea-pig. I publish these observations in the hope that they may contribute to the establishment of Schultz's discovery on a firmer basis. Incidentally some details of the results appear to me to have an important bearing on the theory of the anaphylactic process. I have some hope also, that the very simple method, which I describe, may be found useful by others for certain kinds of investigations, as giving a very clear and objective index of sensitisation in the guinea-pig.

## 2. METHOD

I employed throughout the constant-temperature bath and recording apparatus for isolated organs which Laidlaw and I<sup>26</sup> recently described in another connection. The Ringer solution used was made up on Locke's formula, with the exception that only 0.012 per cent of calcium chloride was used in place of the usual 0.024 per cent. I am by no means certain that this change

<sup>26</sup> Journ. Pharm. and Exp. Therap., iv, p. 75, 1912.

has any advantage. It originated through a mistake of the assistant who made up the solutions. It appeared to me that the isolated organs in the solution with reduced calcium were less liable to acquire a disconcerting spontaneous rhythm, and that the sensitive ones gave a better response to the corresponding protein, and were more readily desensitised by the action of a first dose, than in a solution with the normal calcium proportion.

The observation seemed to be in line with other evidence as to the protective value of calcium against anaphylactic symptoms, but I attach no significance to it at present, as it depended on general impression only, and no deliberate experiments on the point have yet been conducted.

I have tried various plain muscle tissues from the guinea-pig and have found none to approach a uterine horn from a virgin in regularity and delicacy of response. Schultz figures incidentally an experiment with a guinea-pig's uterus; but the figure gives the impression that the organ was taken from a full-grown and probably multiparous animal. It shows an enormous spontaneous rhythm and the effect of horse serum, even in the very high concentration which he employed, is not at all striking. As described in the paper by Laidlaw and myself, the uterus of the virgin guinea-pig, suspended in oxygenated Ringer's solution at 38°C., soon loses the tonus, excited by the exposure incidental to its removal from the body, and characteristically assumes a condition of very low tone, with a small, and fairly regular rhythm. For many of the experiments described in this paper, particularly those illustrating the phenomena of desensitisation and resensitisation *in vitro*, it is essential to use very small and slender uteri. These are obtainable at most seasons from any virgin sow of less than 250 grams weight. When I have required uteri of this size sensitive to horse serum, I have commonly taken survivors from antitoxin standardisations, which had received sufficient excess of toxin to prevent any increase in weight, during the ten days subsequent to injection, necessary for full sensitisation. From such animals I usually obtained uteri of the very slender type required. The horn of such a uterus, when

in its normal condition of full extension in the saline bath, forms a narrow and very delicate ribbon. At certain seasons the uteri from even very young animals, of less than 200 grams, are thicker, appear congested, and show a much more pronounced tendency to rhythm. At the time of writing, in the first week of October, I have failed to find a uterus ideal for the purpose for some weeks past. An association of this heightened activity and congestion with an oestral period is the obvious suggestion, but there seems no indication of a generally prevalent oestrus at the time in the breeding habits of the pigs. However that may be, the difficulty is not encountered during most of the year, if quite young virgins are used.

The uterus has a great advantage, over all other samples of plain muscle which I have tried, in the length of time during which it will retain its vitality and excitability as an isolated organ. For a period of some hours the excitability improves rather than diminishes in the Locke-Ringer solution, and, with the very small type of uterus, it is many hours before the spontaneous rhythm becomes so excessive or irregular as to obscure results.

In experiments with sensitive uteri precautions were in many cases taken to ensure that the plain muscle was perfectly free from blood. The animal was killed by a blow on the head and bled out as completely as possible. The abdomen was widely opened, the rectum tied and cut across, and the whole of the bowels and stomach pulled forwards. The animal was then cut completely across at the level of the kidneys, care being taken to preserve the ovaries. The aorta was dissected out low down, a suitable glass cannula was tied into it, and warm oxygenated Locke's solution perfused through the lower part of the animal for upwards of half an hour, the uterus being meanwhile protected with warm saline pads. The solution passes forwards through the uterine arteries into the anastomosing ovarian arteries and through the series of transverse branches to the whole of the uterus. The vena cava should be inspected and opened if necessary: it occasionally becomes occluded by the crushing of tissues in trans-sectioning the animal. After a few minutes



perfusion the fluid flowing from the cut cava appears to be pure Ringer's solution. By the end of the period of perfusion the whole uterus is washed perfectly white and appears somewhat oedematous from the distension of its vessels with Ringer's solution, of which 0.5 to 1 liter is in each case thus passed through the hinder part of the animal. There seems no reason for supposing that, after such treatment, the plain muscle fibers of the uterus are less free from serum than, say, the washed blood-corpuscles of the typical haemolysin experiment. The method of excision and suspension was as previously described. Saline baths of varying sizes were used in different experiments, those most commonly used holding 20, 50, and 250 cc. respectively. The arms of the straw lever had a ratio of 2 to 1, so that the contractions were recorded double the actual size. The ink-writing point weighed 0.25 gram, giving an effective extending load on the uterus of 0.5 gram, in addition to the excess weight of the longer arm of the light straw lever. Only with the bigger uteri was additional load necessary, and this was then added by hanging a small weight on the lever near the fulcrum.

### 3. THE REACTION OF THE ANAPHYLACTIC UTERUS TO THE ANTIGEN, AND OF THE NORMAL UTERUS TO THE SAME

My method of procedure differs from that of Schultz in a manner dependent on a fundamental difference of conception. Finding that native sera and other protein-containing products have a stimulating action on isolated plain muscle in large doses, Schultz regards the anaphylactic reaction as an exaggeration of this normal response. He, therefore, treats the anaphylactic organ with a dose of the sensitising serum of such dimensions as to cause contraction of the normal control, and is content to show that a larger response usually results. I have already pointed out that his tracings, if they are regarded as accurately indicating the response of tissues in the whole animal, would lead to the expectation that a normal guinea-pig would be as readily killed by injecting 1 cc. of its own serum as a horse-sensitive guinea-pig by injecting 1 cc. of horse serum: and that,

at most, the sensitive pig would be killed rather more readily by horse serum than the normal pig. It seems clear to me that he is confusing two interestingly similar, but none the less fundamentally distinct phenomena; namely, the toxic effect on all animals of large doses of fresh sera and other native proteins, and the specific effect of minute doses of the sensitising protein on the sensitised animal. It is, therefore, essential to the demonstration of the anaphylactic condition, that all experiments should be conducted with a dosage so low, or with preparations so deprived of natural toxicity, that no effect is produced on the normal tissue. The essence of the anaphylactic condition lies in the fact that the tissue responds violently to doses far below the range of those which affect the normal tissue. The normal toxicity of sera is at a maximum just after separation from the clot. It is apparently due to a thermostable substance or substances, which are, however, readily carried down with the coarse coagulum, if serum is boiled without suitable dilution. Keeping the serum for a few days greatly diminishes its toxicity to the normal tissue, without in any way affecting the response of the anaphylactic tissue to the specific antigen. I find that sera a few days old can be added to the bath in the proportion of at least 1 cc. to 250 cc. of Ringer solution, without producing any effect on the normal uterine muscle. In my earlier experiments with sensitised uteri, I made a practice of adding first to the bath a dose of a serum, from a species other than that to the serum of which the guinea-pig had been sensitised, to ensure that the dosage adopted was well below the range of non-specific toxicity. Figure 1, for example, exhibits the response of a small uterus, from a guinea-pig sensitised to horse serum.<sup>27</sup> This uterus was perfused for one hour with Ringer's solution before suspension in the bath. Like other uteri subjected to prolonged perfusion with Ringer, it shows a rather more sluggish rhythm, and becomes less completely extended with suspension, than a similar uterus simply suspended without perfusion. For this

<sup>27</sup> In all figures, except figure 9, the tracing shown is that recorded by an isolated horn of the virgin guinea-pig's uterus. "Perfused" signifies washed by prolonged perfusion with Ringer's solution before excision.

reason such uteri appear, on the whole, to give a less intense reaction with the specific serum: but they also react more sluggishly with stimulant drugs in general, and the effect is presumably a mechanical result of the oedema produced by the perfusion. That it is certainly not due to any loss of specific sensitiveness is abundantly obvious from the fact that such a uterus

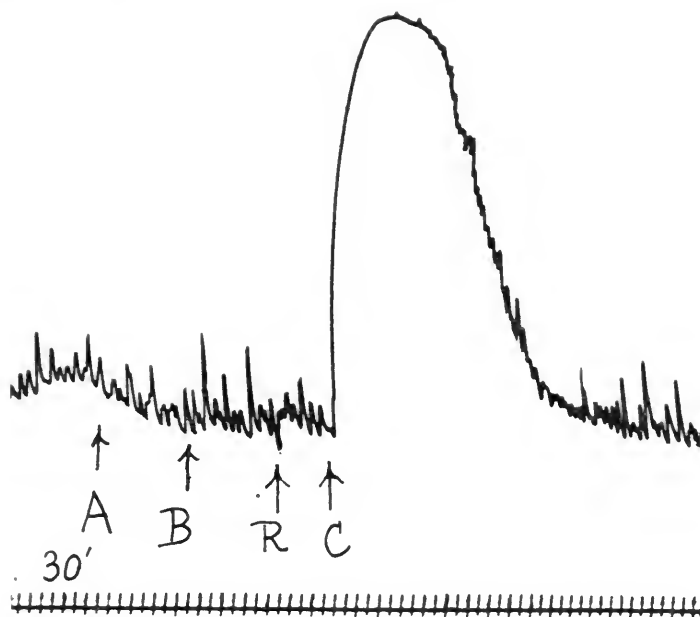


FIG. 1. Sensitisation:  $1/540$  diphtheria antitoxin (horse) + 1 test dose of toxin, fourteen days previously. Perfused. Bath volume 250 cc. At A added 0.5 cc. sheep serum. At B added 0.5 cc. cat serum. At R (in this and all other figures) run off the fluid in the bath, wash out, and replace by clean Ringer's solution. At C added 0.1 cc. horse serum.

will react, as definitely as one not perfused, with the minutest dosage (cf. fig. 6). It will be seen from figure 1 that this uterus gives no reaction of any kind when 0.5 cc. of sheep serum or 0.5 cc. of cat serum is added to the 250 cc. bath, but immediately responds to 0.1 cc. of horse serum with maximal tonus. By "immediately" I mean that there is no true latent period measurable under the conditions of the experiment. The length of

the *apparent* latent period is shown in figure 2, taken at a faster rate, the lever being moved slightly by hand as the serum is added to the bath, and the rise of tonus following this indication

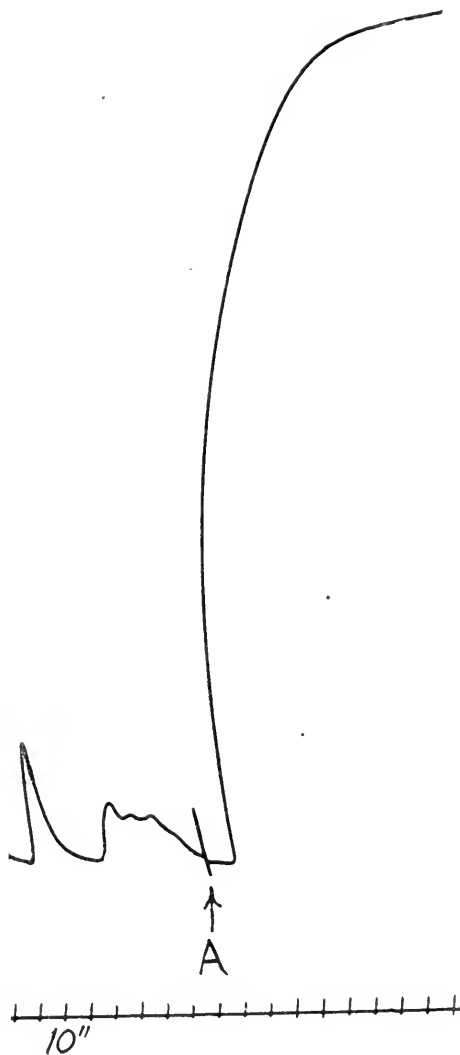


FIG. 2. Sensitisation:  $1/480$  diphtheria antitoxin + 1 test dose toxin, fourteen days. Not perfused. Bath volume 250 cc. Tracing taken with faster drum than that used in figure 1, to measure rapidity of onset of effect. A, 0.5 cc. horse serum.

within ten seconds. The serum is added at a point remote from the immersed organ, and even when brought into contact with it by the stirring action of the oxygen-bubbles, has to reach the muscle fibers through the peritoneal covering and connective

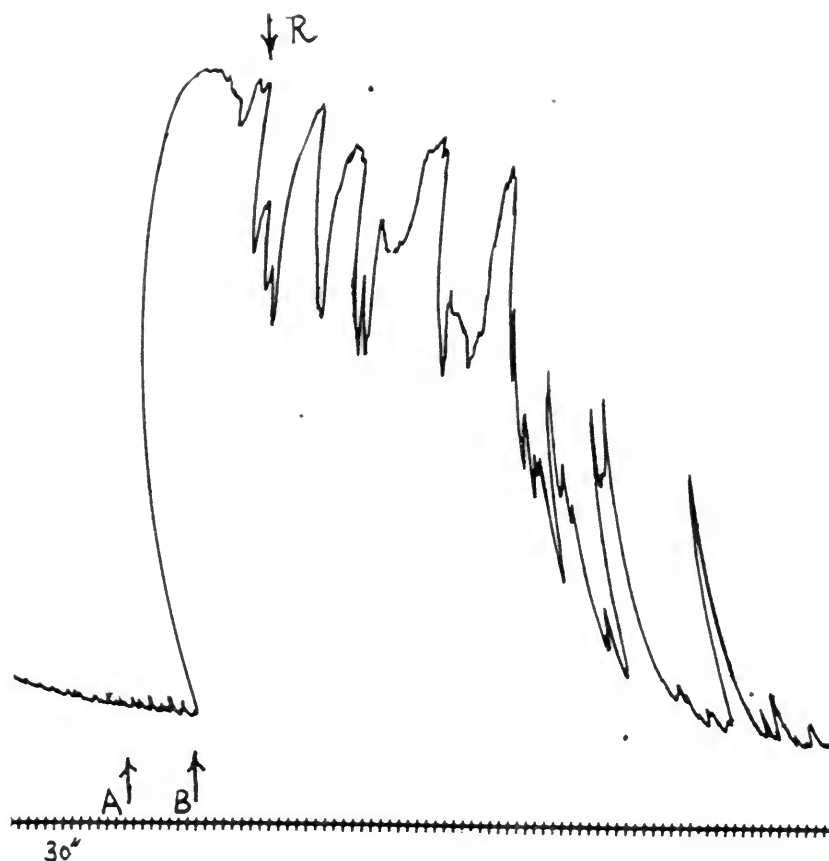


FIG. 3. Sensitisation: 0.1 cc. sheep serum, thirteen days. Not perfused. Bath volume 250 cc. A, 0.5 cc. horse serum. B, 0.5 cc. sheep serum.

tissue. It is obviously impossible to assign any true latency to the effect under such conditions. The action appears with as little delay as that of most drugs applied under the same conditions, and with less than that of many.

Figure 3 shows a tracing from the uterus of a guinea-pig sensitised to sheep serum. This is entirely unaffected by 0.5 cc. of horse serum, but gives the characteristic response to sheep serum. Figure 4 shows a similar tracing from the uterus of a guinea-pig sensitised to egg-white: this again is unaffected by 0.5 cc. of horse serum, but gives the characteristic response to 0.1 cc. of egg-white.

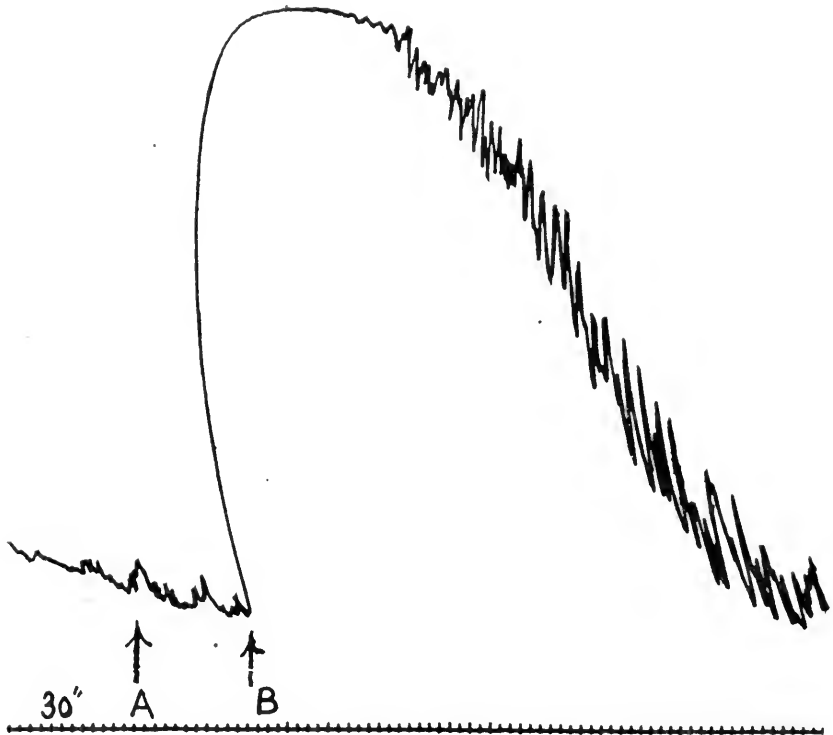


FIG. 4. Sensitisation: 0.1 cc. egg-white, twelve days. Not perfused. Bath volume 250 cc. A, 0.5 cc. horse serum. B, 0.1 cc. egg-white.

I soon found, however, that doses of this order, even though already well below the limit of the normal toxicity of the preparations, were far larger than necessary for the production of the specific response of the anaphylactic plain muscle. Figure 5 shows the reaction of a uterus from a guinea-pig sensitised to

horse serum when 0.0001 cc. of the serum is added to the bath containing 50 cc. of Ringer solution, i.e., to a concentration of 1 in 500,000 horse serum. The uterus from another sensitive guinea-pig, after prolonged perfusion with Ringer's solution, yielded the tracing shown in figure 6, giving, as will be seen, no reaction with the control dose of Ringer's solution or with 0.00001

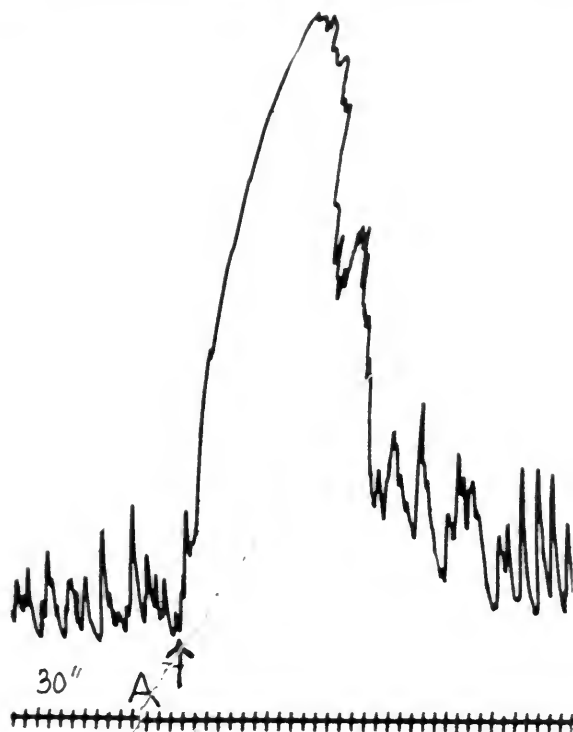


FIG. 5. Sensitisation: 1/300 cc. diphtheria antitoxin + 1 test dose toxin, fourteen days. Not perfused. Bath volume 50 cc. A, 0.0001 cc. horse serum (i.e., 1 : 500,000).

cc. (1 in 5,000,000) horse serum, but a quite definite, though far from maximum response with 0.00005 cc. (1 in 1,000,000). This represents a very high degree of sensitiveness. Individual differences of sensitiveness are observed in uteri from animals sensitised in the same way, just as reinjection with a given dose produced symptoms of varying severity in a series of animals

sensitised in a uniform manner, and tested after the same interval. It may be taken as a rule, however, that the uteri of animals sensitised with a small injection of horse serum, and tested after a full incubation period of twelve days or more, will give a large response to horse serum in dilutions of upwards of 1 in 100,000.

It might fairly be argued that the reaction to these minute

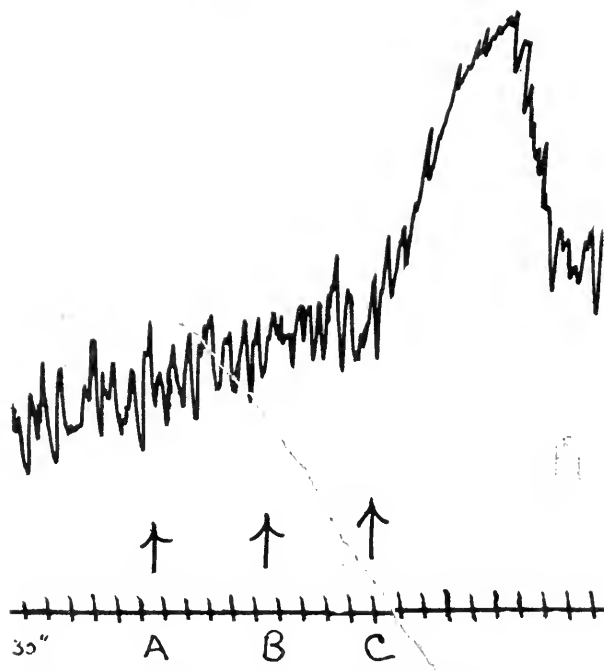


FIG. 6. Sensitisation:  $1/800$  cc. diphtheria antitoxin + 1 test dose toxin, fourteen days. Perfused (800 cc.). Suspended in 50 cc. A, 1 cc. Ringer's solution. B, 0.00001 cc. horse serum (i.e., 1 : 5,000,000). C, 0.00005 cc. horse serum (i.e., 1 : 1,000,000).

doses, is, in reality, an expression of enormously exaggerated sensitiveness to that constituent of the antigenic serum which, in large doses, excites the normal plain muscle. In that case, however, any process which reduces the normal toxicity of the serum should raise the dose necessary to excite the anaphylactic muscle. I can find no indication at all that this is the case. On the contrary, a neutral isotonic solution, containing 15 per cent



of horse serum protein, made by a modification of Gibson's antitoxin-concentration method, for which I am indebted to my colleague Dr. Walpole, has no perceptible action on the normal uterus in a dilution of 1 in 50, beyond which I have not tested

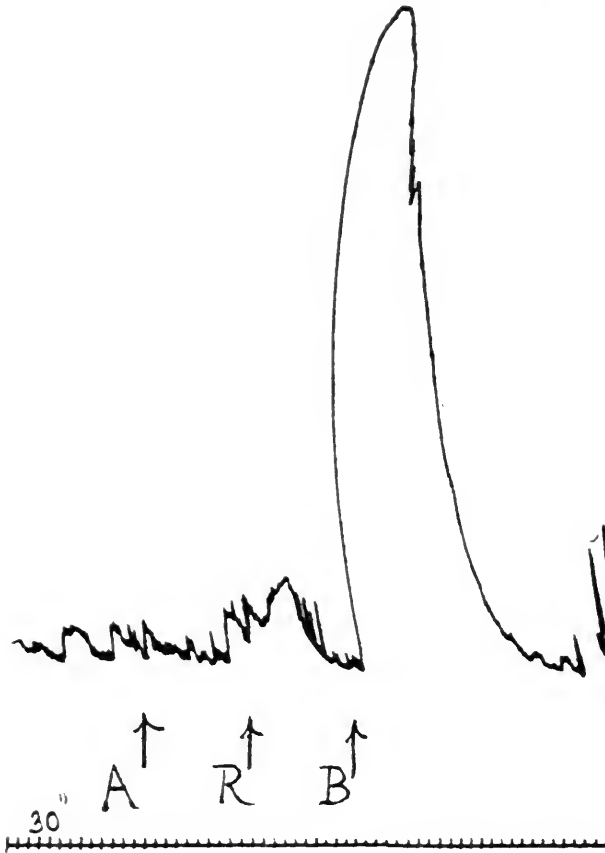


FIG. 7. Normal uterus. A, 1 cc. 15 per cent purified horse globulin. B, 1 cc. fresh guinea-pig serum.

it. Yet this preparation excites the anaphylactic muscle in dilutions of the same order as are effective in the case of the normal serum. Figure 7 shows the result of testing this preparation on a normal uterus. At A, 1 cc. of the horse globulin solution was added to the 50 cc. of Ringer solution in the bath.

At *R* the solution was changed for fresh Ringer. At *B*, 1 cc. of fresh guinea-pig serum was added, the response showing that the uterus was quite responsive to the normal toxic constituent of serum at this dilution. Figure 8 shows the response of a horse-sensitive uterus to the same solution of horse globulin. The bath again held 50 cc. of Ringer solution. At *A*, 0.00005 cc. of the globulin solution was added, making a dilution of 1 in 1,000,000 of the solution or 1 in 6,500,000 of globulin. This having no effect, a second dose of 0.0005 cc. was added at *B*, making 1 in 100,000 of globulin solution, or 1 in 660,000 of dry globulin. This causes a powerful reaction, showing that the sensitive uterus responds to this preparation in dilutions of the same order as the highest dilutions of normally toxic serum which suffice to excite the average horse-sensitive uterus. Further and even more convincing evidence, of the distinct nature of the anaphylactic response, from the normal toxic effect of fresh serum, is afforded by the rest of this tracing, which, however, may be more conveniently discussed in the next section.

#### 4. EFFECT ON THE ANAPHYLACTIC UTERUS OF REPEATED DOSES OF THE ANTIGEN

When the sensitised uterus is of the thin type which I have described as suitable for these experiments, the effect of a single dose, if sufficient to cause a maximal response, is to remove the sensitiveness completely, so that further doses are quite without effect, provided that non-toxic preparations of protein are used, or that the dose, if preparations of natural toxicity are used, is kept below the threshold of action on normal tissues. With larger, thicker uteri it is more difficult to effect a complete and permanent desensitisation. This is easy to understand, if it is remembered that the solutions are only applied to the outside of the organ, and that proteins cannot be expected to find their way rapidly through any thickness of muscle. Even uteri from full-grown virgin guinea-pigs, however, give to a second dose a trifling response as compared with that evoked by the first, provided that the latter is not too small. This process of desensiti-

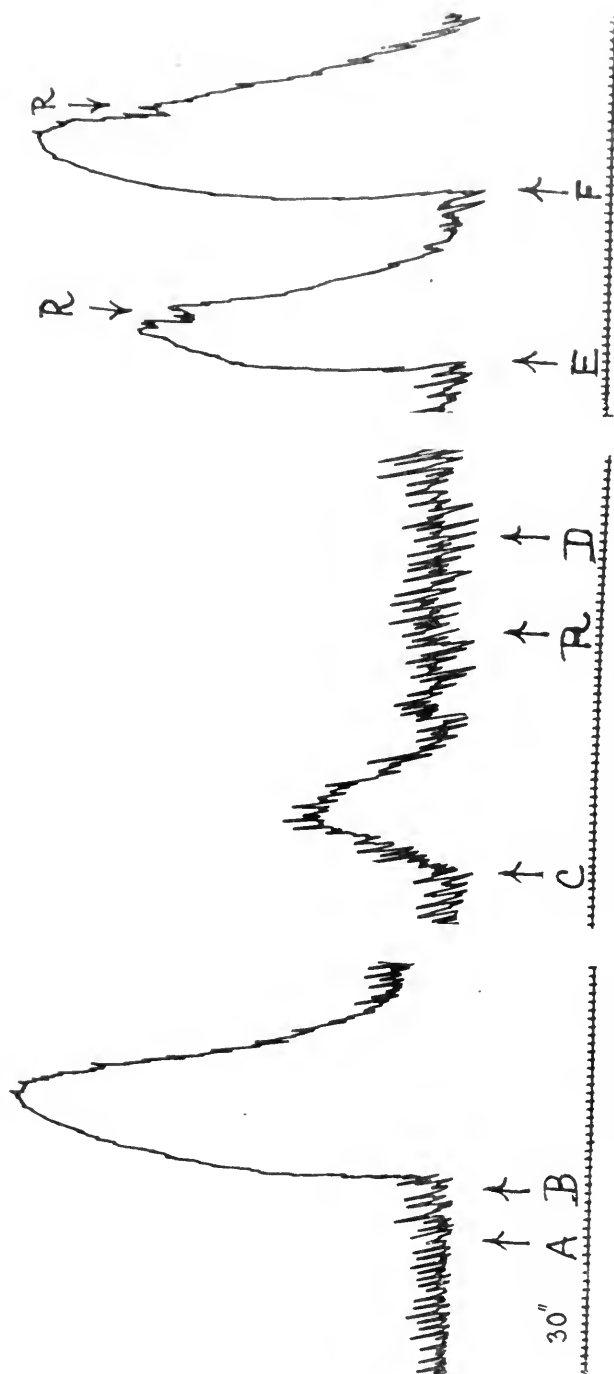


FIG. 8. Sensitisation: 1/200 cc. diphtheria antitoxin + 1 test dose toxin, fourteen days. Perfused. Bath volume 50 cc. A. 0.0005 cc. 15 per cent horse globulin. B. 0.0005 cc. of the same. C (after change of Ringer). 0.05 cc. of the same. D, 1 cc. of the same. E' (after change of Ringer), 1 cc. horse serum, twenty-four hours old. F, 1 cc. perfectly fresh guinea-pig serum.

sation, which obviously corresponds to the "anti-anaphylaxis" of the whole animal, is illustrated in several of the figures (cf. figs. 8, 12, 15, 17, 21). It is well shown in figure 8, which is particularly instructive, in that it clearly demonstrates the reason why Schultz failed to observe this anti-anaphylactic condition, and obtained repeated contractions with successive large doses. After the effective, but not yet maximal dose of 0.0005 cc. of 15 per cent horse globulin solution at *B*, the Ringer was changed, during the period corresponding to a section of tracing removed to save space. Then, at *C*, 0.05 cc. of the same preparation produced a comparatively small but distinct effect. This concentration was sufficient to effect a complete desensitisation, so that even 1 cc. (1 in 50 of the solution, 1 in 330 of globulin) at *D* produced no trace of effect. After a further change of solution, 1 cc. of horse serum, twenty-four hours old, was added at *E* and produced a large contraction. Now normal horse serum contains only 8 to 9 per cent of protein, so that the dose of protein given at *E* is little more than one-half of that given at *D*. The uterus then, though completely desensitised to horse protein, can still respond, like a normal uterus, to the toxic constituent of fresh serum. That this response has nothing to do with horse protein, or with anaphylaxis, is further proved by the fact that, after a further change of solution, the uterus gives a still larger reaction to 1 cc. of fresh guinea-pig serum, added at *F*.

It seemed practically certain, therefore, that the contractions observed by Schultz, with doses subsequent to the first, were all of the nature of the reaction of a normal tissue to the toxic constituent of fresh serum, the originally anaphylactic tissue being presumably reduced, by the first very large dose (1 in 10 horse serum), to the condition of a normal tissue with respect to pure horse protein. There was just a possibility, however, that the muscle of the intestine, as used by Schultz, would behave differently in this respect from that of the uterus. I therefore made a few experiments on this point with intestinal loops from sensitive guinea-pigs. The result was uniform and definite, though I have always found the intestinal muscle far less specifically sensitive than that of the uterus. Figure 8 illustrates a

typical result. It will be seen that the sensitised muscle gives a clear response to the first dose of 0.5 cc. of 15 per cent horse globulin, added at *A* to the 50 cc. of Ringer solution in the bath.

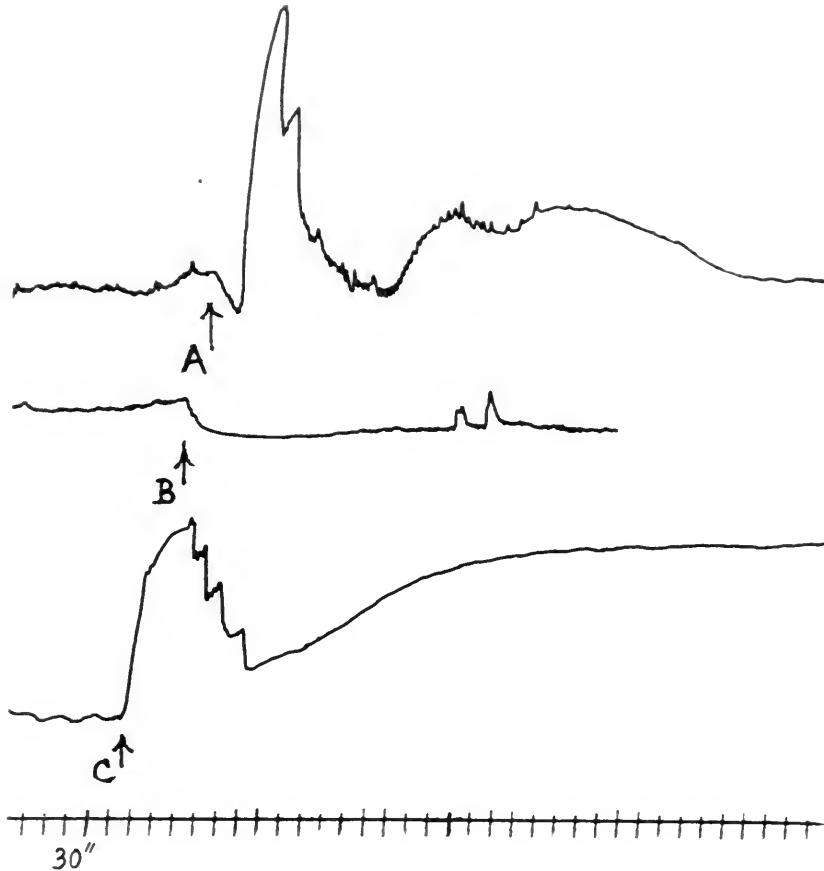


FIG. 9. Segment of small intestine, 40 mm. long, from sensitised guinea-pig. Ratio of lever arms 1 : 5. Sensitisation: 1/400 cc. diphtheria antitoxin + 1 test dose toxin, seventeen days. Bath volume 50 cc. *A*, 0.5 cc. 15 per cent horse globulin. *B*, second similar dose (after change of Ringer). *C*, 0.5 cc. *fresh* guinea-pig serum (after further change of Ringer, with thorough washing).

After changing the solution, a second similar dose is added at *B*, and produces no trace of contraction, the only effect being a small relaxation. The intestinal muscle is therefore completely

desensitised to horse protein. It is still capable, however, of giving a marked reaction to fresh guinea-pig serum, added, after thoroughly washing away the horse protein, at *C*.

### 5. MULTISENSITISATION

Rosenau and Anderson<sup>28</sup> have shown that guinea-pigs may be anaphylactic to three proteins at the same time, and that a non-fatal injection of one of the sensitive preparations is followed by anti-anaphylaxis to that one only, the animal subsequently giving typical reactions to the others. My own experiments on such multisensitisation gave rather less clear results, and there are some points which might repay further investigation. Only four such experiments were made, the sensitising antigens being in all cases injected at the same time under the skin, either in mixed solution, or separately. Three guinea-pigs each received a mixture containing 1/30 cc. of horse serum, 1/30 cc. of sheep serum, and 1/15 cc. of egg-white. They were tested seventeen to nineteen days subsequently. The uteri of all were sensitive to all three antigens, three successive reactions being obtainable. The sensitiveness to horse serum was in all three cases comparatively low. On the other hand the uterus desensitised completely to sheep serum still gave to horse serum a definite, though small response, and, subsequent to this, a maximal reaction to egg-white (fig. 10). If however, egg-white were given first, the uterus subsequently gave no definite response to either of the sera. In the case of the fourth guinea-pig an attempt was made to sensitise simultaneously to the sera of horse, sheep, cat, dog, rabbit and man, and to egg-white, 0.1 cc. of each being injected hypodermically. Thirteen days later the uterus was tested. There was evidence in this case also of non-specific desensitisation, while the sensitiveness to any one antigen was not of a high order. Since only two experiments on one animal could be made by my method, one with each horn of the uterus, it was not possible to discover whether sensitiveness existed to any antigen except that tested first on each horn. The

<sup>28</sup> Journ. of Infect. Dis., iv, p. 552, 1907.

first horn, suspended in 250 cc. Ringer's solution, was tested first with egg-albumen, 0.1 cc. of a 15 per cent solution of the protein, crystallised by Hopkins' method, being added to the bath, so that the proportion of protein present was about 1 in 16,000. A typical response was obtained. When this had subsided the uterus was tested with 0.1 cc. to 0.5 cc. doses of the various sera

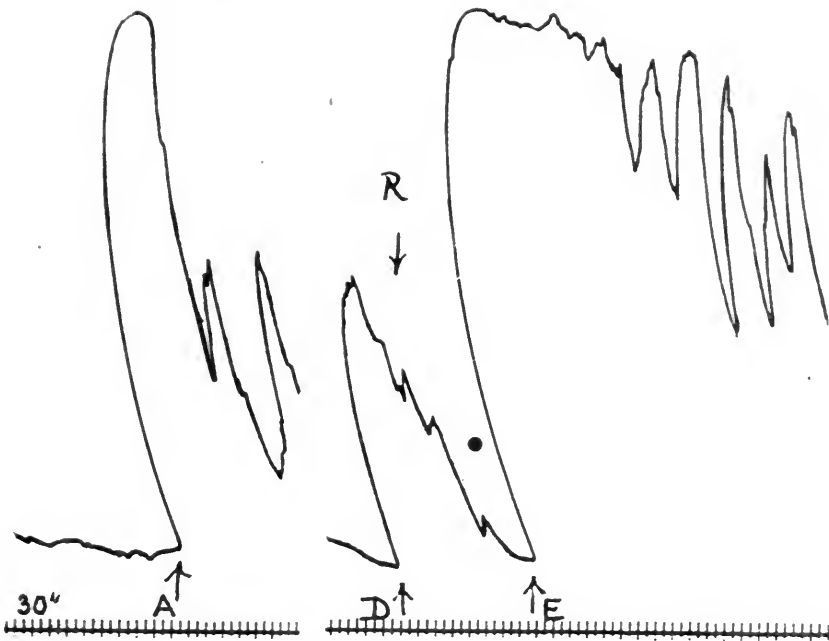


FIG. 10. Sensitisation: 1/15 cc. egg-white + 1/30 cc. horse serum + 1/30 cc. sheep serum, nineteen days. A, 0.5 cc. sheep serum. After changing the solution and giving further doses of sheep serum, to which there was no response, D, 0.5 cc. horse serum. E, 0.1 cc. egg-white.

in succession, but gave no definite response to any of them. The other horn was then suspended, and responded, though not maximally, to 0.5 cc. of sheep serum, but afterwards gave no contraction with 0.5 cc. of human serum or 0.2 cc. of the solution of crystallised egg-albumen, i.e., with double the dose which acted as an efficient initial stimulus to the first horn.

There are two points here which are deserving of further investigation. In the first place it appears that a guinea-pig receiving small simultaneous injections of several different sera acquires to none of them the high degree of sensitiveness which, after the same period, might be predicted as the result of injecting any one of them singly. Possibly a more extended incubation period is necessary under such conditions. In the second place, it would appear that desensitisation to one antigen is not wholly without effect on sensitiveness to others, though the result shown in figure 10 indicates that there is some degree of independence. Apparently in the phenomenon of desensitisation, as studied on the isolated uterus, there is some other factor than that of specific saturation of antibody. If this were the case it might be expected that this non-specific factor would be more prominent when the sensitiveness was of a comparatively low order, as in the attempt to sensitise simultaneously to many antigens.

Incidentally it is of interest to note that pure egg-albumen, crystallised by Hopkins' method, is a perfectly efficient stimulus to plain muscle from a guinea-pig sensitised to egg-white, though, as Schultz observed, it has no effect on that of the normal animal.

#### 6. REACTION OF THE UTERUS FROM GUINEA-PIGS IMMUNISED TO HORSE SERUM

It is stated by Schultz that the intestinal plain muscle of guinea-pigs immunised by repeated injections of horse serum shows, when isolated, a supersensitiveness similar to that exhibited by plain muscle from anaphylactic pigs. My experiments confirm this observation and bring out an additional point of some theoretical interest. We have seen that an adequate dose of the sensitising protein effectively desensitises the anaphylactic muscle, and there can hardly be a doubt that this is the main factor in the condition of "anti-anaphylaxis," immediately following recovery from a non-fatal reinjection. But, as Rosenau and Anderson, and Anderson and Frost,<sup>29</sup> in particular, have

<sup>29</sup> Hygienic Laboratory Bulletins Nos. 29, 45 and 64, Washington, 1906, 1908 and 1910.



emphasised, when repeated injections are given to the animal thus desensitised, the condition of anti-anaphylaxis merges into one of true immunity, so that a considerable interval may be made in the series of injections without the following injection causing any serious symptoms. By use of the isolated organ it is easy to show that this condition of immunity may co-exist with a well-marked sensitiveness of the plain muscle. Some degree of sensitiveness is already apparent at a comparatively early date after the last injection of the series. Figure 11 shows

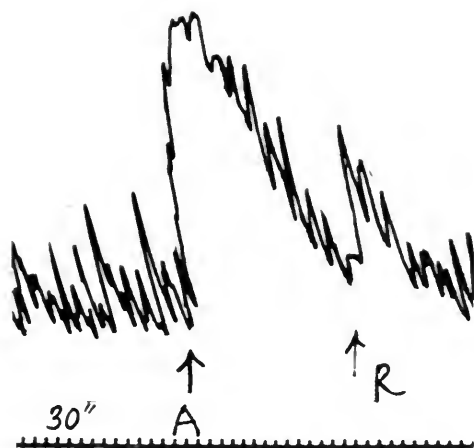


FIG. 11. Guinea-pig immunised to horse serum. Three injections (see text). Tested five days after the last. Perfused. Bath volume 250 cc. A, 0.5 cc. horse serum.

the definite, though weak response of the uterus from a guinea-pig which was treated as follows: On November 22 it received 1/500 cc. of diphtheria antitoxic (horse) serum + 1 test dose of toxin; on November 29, i.e., before development of full anaphylaxis, it received 4 cc. of horse serum hypodermically, and showed a trace of reaction but quickly recovered; on December 6 it received 4 cc. of horse serum intraperitoneally without showing any trace of symptoms. On December 11, i.e., only five days after the last injection, the uterus was perfused free from blood in the usual way and suspended. One-half cubic centi-

meter of horse serum, added to 250 cc. of Ringer solution, produced the small effect shown in figure 11. After a longer period since the last injection, but still at a time when the animal, according to all experience, would prove almost indifferent to injections of horse serum, the uterus, free from blood, shows a high degree of sensitiveness.

Figures 12 and 13 illustrate this and another point of some interest. They are from the two horns of one of a number of guinea-pigs, which were reinjected during the incubation period following a first injection, and then received a series of intraperitoneal injections of 1 to 3 cc. of horse serum (ten injections in all) at intervals of from three to fourteen days. Twenty-four days after the last injection several were again injected as controls with 5 cc. of horse serum intraperitoneally and showed no sign of reaction in some cases, and a very slight trace in others. The pig from which the uterus gave figures 12 and 13 was killed, bled and perfused as usual. The first horn, suspended in 250 cc. Ringer's solution, was given 0.5 cc. of sheep serum as a control. Contrary to all expectation it reacted strongly (fig. 12, *A*). After changing the Ringer's solution 0.1 cc. of horse serum was given at *B* and produced a yet higher, though less persistent contraction. The rest of the tracing shows that the uterus, though now desensitised to sheep serum, was still capable of modified reaction to 0.5 cc. horse serum, the first dose (1 in 2500) being inadequate, in this case, to effect complete desensitisation. The other horn was then suspended in the same volume and given, as a first dose, 0.5 cc. horse serum (1 in 500). This produced a typical maximal response (fig. 13), and the uterus, after changing the solution, was found to be desensitised to sheep serum as well as to horse serum. Other guinea-pigs from the same series, tested at the same time, gave practically identical results. A careful investigation revealed no possibility of accident by which any of the samples of horse serum used in the immunising process could have become contaminated with sheep serum. It must be concluded, therefore, that the sensitiveness of the plain muscle, in a guinea-pig immunised by a long series of injections, is less sharply specific to the immunising protein, than that of

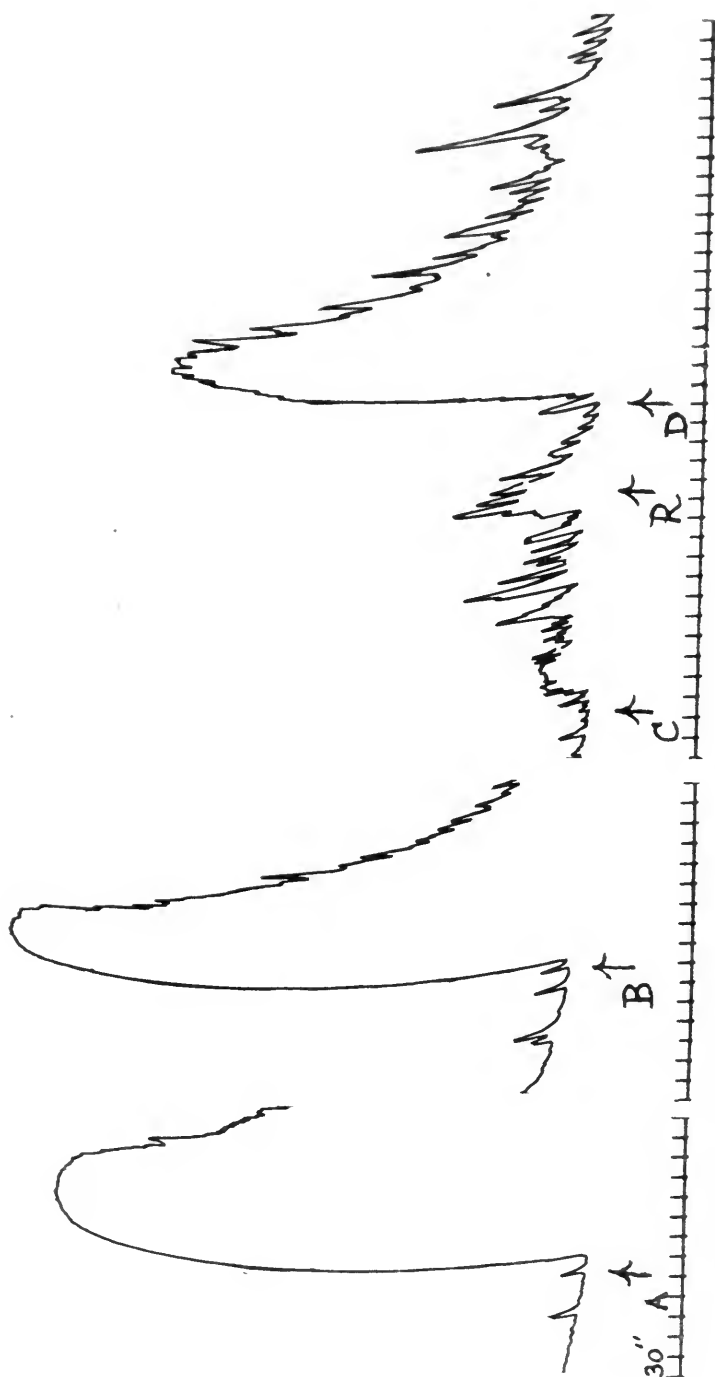


FIG. 12. Guinea-pig immunised to horse serum. Ten injections (see text). Tested twenty-four days after last injection. Perfused. Bath volume 250 cc. A, 0.5 cc. sheep serum. B (after change), 0.1 cc. horse serum. C (after further change), 0.5 cc. sheep serum. D, 0.5 cc. horse serum.

plain muscle from the anaphylactic guinea-pig to the sensitising protein. The specificity becomes, in other words, quantitative instead of qualitative. Unfortunately no experiments were made with non-specific proteins other than sheep-serum.

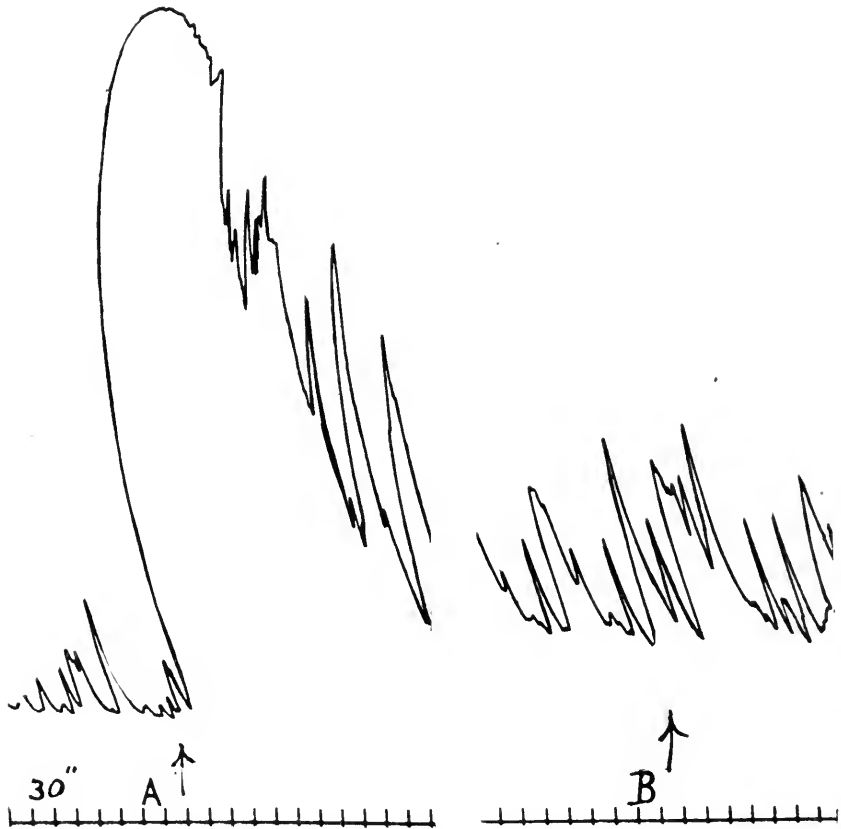


FIG. 13. Same experiment as figure 12. Second horn. A, 0.5 cc. horse serum. B (after change), 0.5 cc. sheep serum.

Several guinea-pigs of this series were kept for a longer period after the last injection, being tested after an interval of three months. The controls, tested with intraperitoneal injections, were still immune to horse serum, and the uteri, taken from others, had largely lost their sensitiveness, giving only a small

reaction to 1 in 500 horse serum and a still smaller, though quite definite reaction to the same dilution of sheep serum. Whether the sensitiveness would fade still further, or increase again, after a yet longer interval, is a point worth further investigation. This gradual desensitisation of the tissue in the immune animal, apart from its protection by circulating antibody, is, I believe, a new point, and contrasts with the life-long sensitisation by a single injection, as described by Rosenau and Anderson.<sup>30</sup>

#### 7. PASSIVE SENSITISATION

It was shown by Gay and Southard<sup>31</sup> that injection of serum from an anaphylactic guinea-pig into a normal guinea-pig produced an anaphylactic condition in the latter. This they wrongly interpreted as an active anaphylaxis produced by a trace of horse serum remaining in the circulation of the donor. Otto<sup>32</sup> showed that the transferred sensitiveness could already be detected twenty-four hours after the injection, and was, therefore, a genuinely "passive" anaphylaxis. Doerr and Russ, Anderson and Frost, among others, have shown that serum from immunised animals is more effective than that from sensitive animals in producing passive sensitisation. It was found, however, that the sensitisation was not immediate. Otto gave twenty-four hours as the necessary interval. Doerr and Russ, by using intravenous injection, could detect sensitiveness already after four hours. This incubation period of the passive transfer seems to be peculiar to guinea-pig. Friedemann<sup>33</sup> found immediate reinjection necessary for detecting passive anaphylaxis in the rabbit, and, according to Richet<sup>34</sup> and to Manwaring,<sup>35</sup> transfusion from an anaphylactic dog to a normal one results in immediate sensitisation of the latter, though this was not observed by Pearce and

<sup>30</sup> Hyg. Lab. Bulletins No. 50, Washington, 1909.

<sup>31</sup> Journ. of Med. Research, xvi, p. 143, 1907.

<sup>32</sup> v. Leuthold. Gedenkschr., i, p. 1, 1906, Münch. med. Wochenschr., liv, p. 1665, 1907.

<sup>33</sup> Zeitschr. f. Immunit., ii, p. 591, 1909.

<sup>34</sup> L'Anaphylaxie, Paris, 1912.

<sup>35</sup> Zeitschr. f. Immunit., viii, p. 1, 1910.

Eisenbrey.<sup>36</sup> The incubation period in the guinea-pig has been interpreted as the interval necessary for fixation of the specific antibody to the tissues. If this view were correct, and if the fixation were irreversible, then the blood-free, perfused uterus, from a passively sensitized guinea-pig, should show sensitiveness when isolated, like that from the actively sensitised pig. Figures 14 and 15 show that this is indeed the case. The guinea-pig

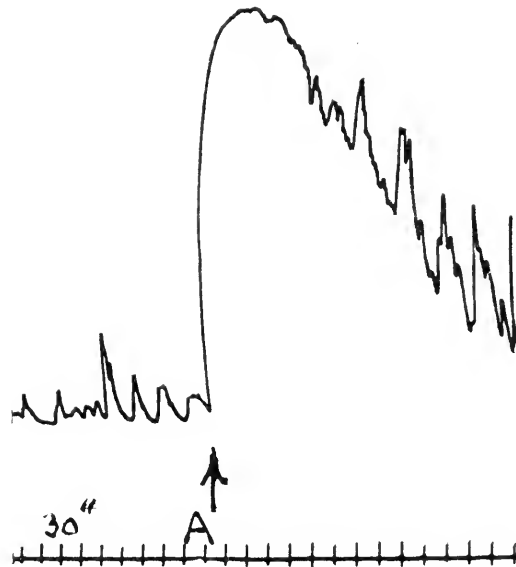


FIG. 14. Passive sensitisation. Serum from guinea-pigs immunised to horse serum (see text). Perfused. Bath volume 50 cc. At A 0.05 cc. horse serum.

whose uterus gave the reaction illustrated in figure 14 was sensitised by injection of serum from two guinea-pigs of a series treated with repeated injections of horse serum, in which the controls showed immunity to large injections. 4 cc. of such serum were given intraperitoneally on one day, 5 cc. on the next, and on the third the pig was bled, the uterus perfused, and the test made as usual. The result (fig. 14) confirms the statement that the serum from immunised guinea-pigs is

<sup>36</sup> Journ. of Infect. Dis., vii, p. 565, 1910.

effective in producing passive sensitisation, and shows that, as in active sensitisation, the plain muscle of the uterus is sensitised. The guinea-pig giving the uterus, of which the reaction is illustrated in figure 15, was sensitised by intraperitoneal injection, on successive days, of 4 cc. and 5 cc. of mixed serum from actively anaphylactic guinea-pigs, which had survived antitoxin standardisations, made ten to sixty-four days previously. The experiment was performed on the day following the second injection. It will be seen that the passively sensitised uterus is as readily desensitised by a first dose as the actively anaphylactic organ. a second dose of horse serum, at *B*, being quite without effect. At the same time it will be apparent, from the size of the reaction to the first dose, that the plain muscle from animals thus passively sensitised has a lower order of sensitiveness than that from animals in which active anaphylaxis is fully developed. This discovery that the plain muscle is sensitised, in passive as in active anaphylaxis, gives us the opportunity of examining further the nature of the processes concerned in passive sensitisation. If the process consists simply of adhesion of antibody to, or its incorporation into the plain muscle cells, it should be possible to effect a passive sensitisation of the surviving organ *in vitro*. The process would, in fact, be comparable to the sensitisation of washed red cells with haemolytic amboceptor.

My first attempts were directed to the resensitisation of uteri which, originally sensitive, either actively or passively, had been desensitised *in vitro* by an adequate dose of antigen. Such a desensitisation, of the uterus from a passively anaphylactic guinea-pig, is shown in figure 15 and has already been referred to. After a second dose, at *B*, had shown that desensitisation was complete, the uterus and bath were well washed with many changes of Ringer's solution, and 2 cc. of fresh serum from a sensitive guinea-pig were then added to the clean Ringer's solution in the bath, which in this case held 20 cc., so that the concentration of sensitive serum was 10 per cent. The uterus, which gave the usual marked response to the fresh, toxic serum, was left in this 10 per cent dilution at 38°C. and with continuous oxygenation for two and one-half hours. Then it was again thor-

oughly washed with many changes of pure Ringer, and, as seen in figure 16, regained a practically normal tone and rhythm. At *E* it was again tested with 0.05 cc. of horse serum (1 in 400) and gave a quite definite response, though not so large as its original response to the same dose (fig. 15, *A*). After a further change of solution another dose (at *F'*) showed that the uterus

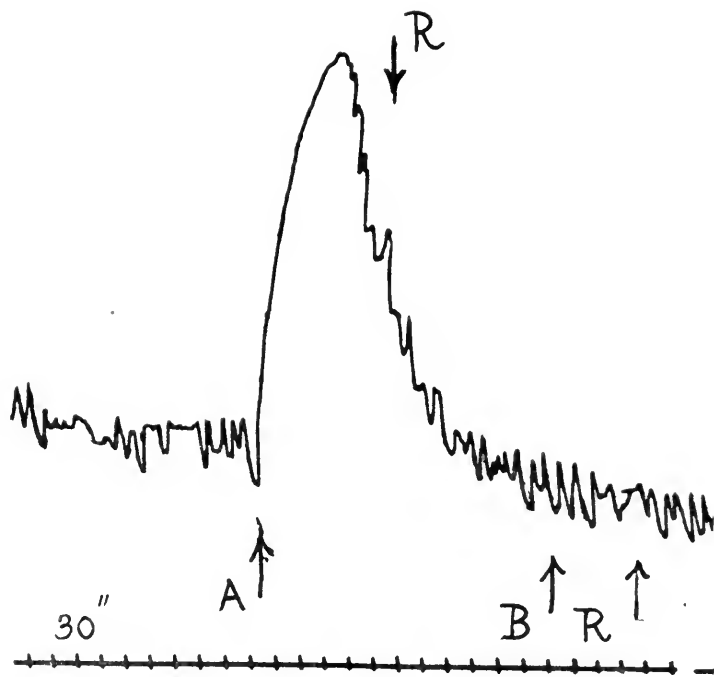


FIG. 15. Passive sensitisation with serum from guinea-pigs anaphylactic to horse serum (see text). Perfused. Bath volume 20 cc. *A*, 0.05 cc. horse serum. *B*, the same.

was again desensitised. Figures 17 and 18 illustrate a similar experiment with an actively sensitized uterus. Figure 17 shows the initial reaction to horse globulin followed by complete desensitisation. After three hours in contact with 7 cc. of sensitive serum, added to the 50 cc. bath, it was again tested, and with 0.05 cc. of the globulin solution (i.e., 1 in 1000 of the solution or 1 in 6500 of globulin) gave the quite definite reaction shown in



figure 18. It will be seen that there is rather a prolonged delay in the reaction to this dose, and this is a common feature in the response to very small doses, or to doses which, on account of partial desensitisation, are *relatively* very small. The resensitisation attained *in vitro* was, therefore, not of a high order; but it must be remembered that the time allowed for contact with the sensitive serum was below the minimum found necessary

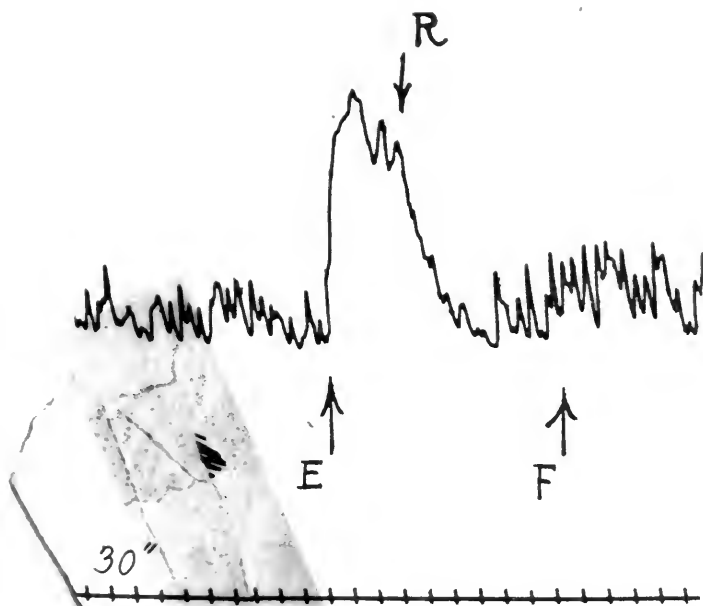


FIG. 16. Same experiment as in figure 15. After resensitisation *in vitro* by soaking for two and one-half hours in 10 per cent serum from horse sensitive guinea-pigs. *E*, 0.05 cc. horse serum. *F*, the same.

when injection is made into the whole animal. But longer contact with sensitive serum in the bath lowered the vitality and general responsiveness of the plain muscle; so that the effect in three hours, though not yet fully developed, is probably the best attainable under the conditions. After shorter contacts, of one to two hours, I obtained no certain evidence of resensitisation.

To make the significance of the effect certain it was further necessary by control experiments to exclude the possibility of spontaneous resensitisation, in the absence of sensitive serum. Figures 19 and 20 illustrate the initial response, *A*, of a sensitive uterus to 0.5 cc. of horse serum in 250 cc. (1 in 500), and the persistence of the desensitisation, following this reaction, after

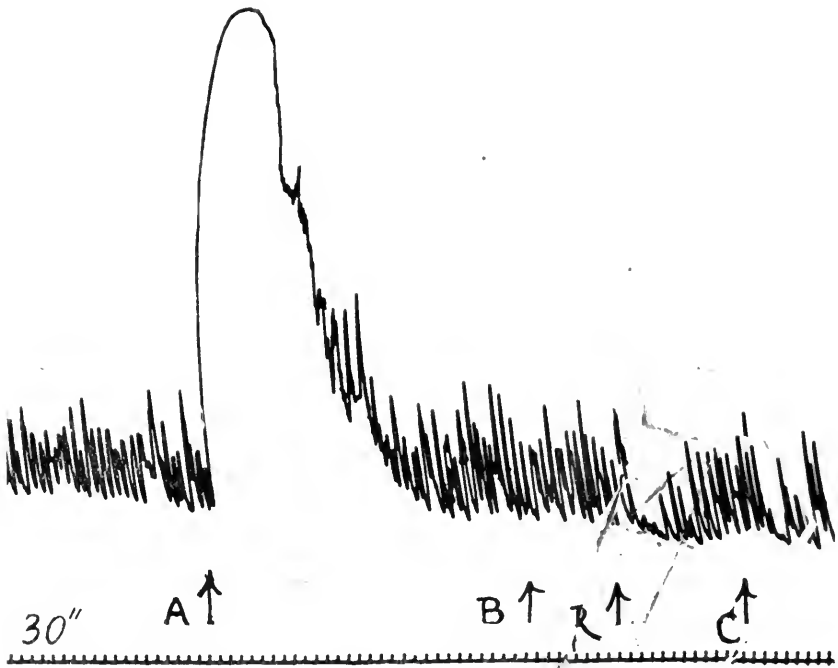


FIG. 17. Sensitisation: 1/450 cc. diphtheria antitoxin + 1 test dose of toxin, ten days. Perfused. Bath volume 50 cc. *A*, 0.02 cc. 15 per cent horse-globulin. *B*, 0.05 cc. of the same. *C*, 1 cc. of the same.

two and one-half hours in frequently changed Ringer's solution; an equal dose, after this interval, being still absolutely ineffective (fig. 20, *B*). In another experiment the originally horse-sensitive uterus, after a first effective and desensitising dose, was left all night in Ringer's solution. On the following morning it still responded well to non-specific stimulants such as  $\beta$ -Iminazolyethylamine, but was still quite indifferent to horse serum. Fig-

ures 21 and 22 illustrate a similar experiment in which the uterus, after desensitisation, was left for three hours in contact with 10 per cent fresh serum from *normal* guinea-pigs. After this treatment it was still quite insensitive to horse serum (fig. 22, *D*) but responded well to  $\beta$ -Iminazolyethylamine (*E*).

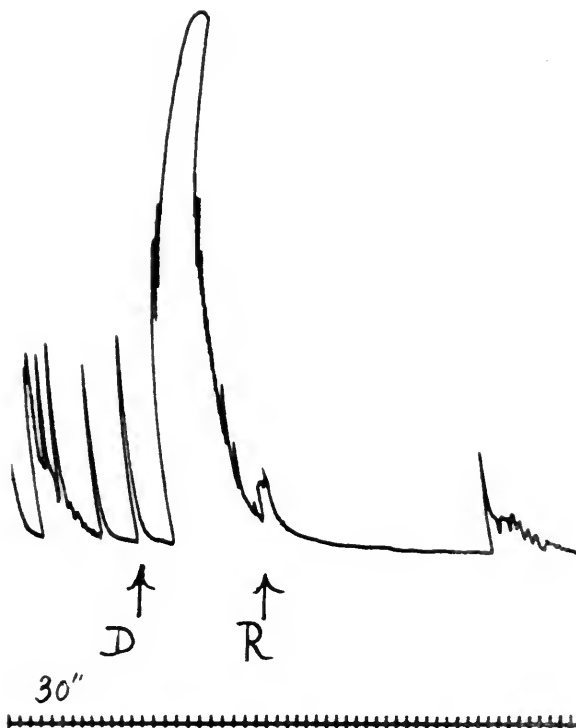


FIG. 18. Same experiment as figure 17. After resensitisation *in vitro* by soaking for three hours in 14 per cent serum from horse-sensitive guinea-pigs. *D*, 0.05 cc. 15 per cent horse globulin.

Having proved the possibility of resensitising *in vitro* a uterus which had been deprived of its original sensitiveness, I expected to be able, by merely suspending for some hours in diluted sensitive or immune serum, to confer passive sensitiveness on the normal organ. I have tried the experiment a number of times, using serum from sensitive or immune guinea-pigs, but have in

no case succeeded in producing a decided sensitiveness by this process. Apparently, for no sufficiently clear reason, the muscle cells which have once held antibody, and have been deprived of it, more readily take it up again, when the whole organ is bathed in a solution containing it, than do muscle cells which have never held it. It seemed possible that mere suspension in the diluted serum did not secure the intimacy of contact

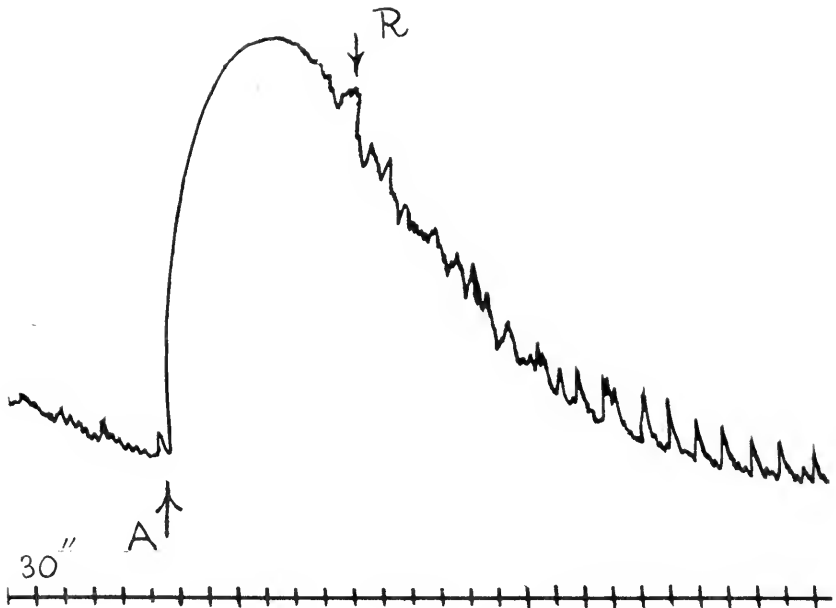


FIG. 19. Sensitisation: 1/1300 cc. antitoxic globulin + 1 test dose toxin, fourteen days. Perfused. Bath volume 250 cc. A, 0.5 cc. horse serum.

necessary for a primary passive sensitisation. I proceeded, therefore, to test the effect of perfusing the normal uterus with sensitive serum, diluted with four parts of Ringer's solution. The apparatus and method were identical with those used for washing the organ with Ringer's solution, the diluted serum being oxygenated in a tall open tube, in which the level was kept constant by means of a Mariotte bottle, warmed by passing through a glass worm in a bath at 40°C., and then passed through

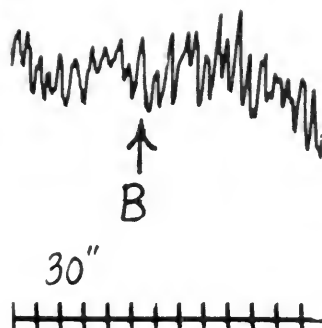


FIG. 20. Continuation of experiment in figure 19. After washing for two and one-half hours in Ringer's solution. *B*, 0.5 cc. horse serum.

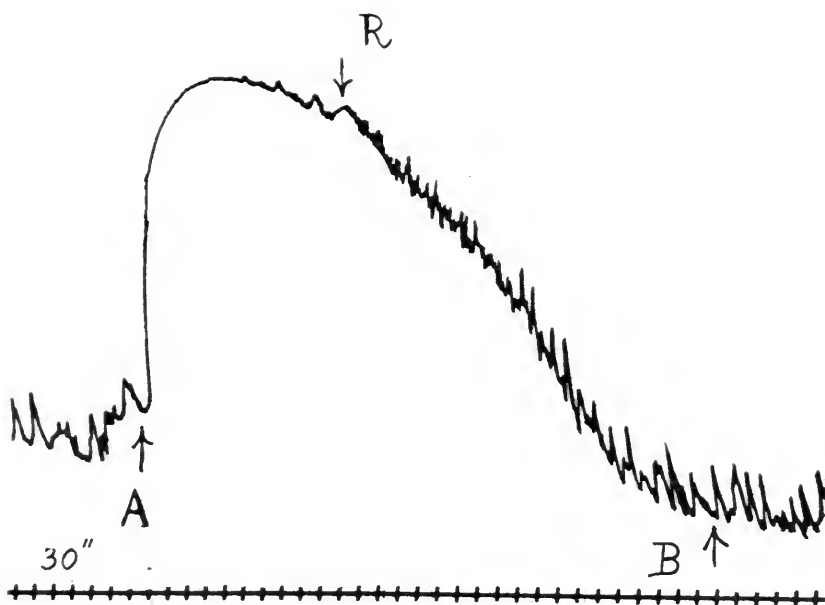


FIG. 21. Sensitisation: 1/420 cc. diphtheria antitoxin + 1 test dose toxin, fourteen days. Perfused. Bath volume 50 cc. *A*, 0.1 cc. horse serum. *B*, the same.

a rubber tube to the cannula in the aorta. Glass-wool filters were inserted at the lower end of the oxygenation tower, and in a bulb, which served also as a bubble trap, just before the aortic cannula. The preparation, consisting of the lower half of the guinea-pig, with all abdominal viscera except uterus and bladder

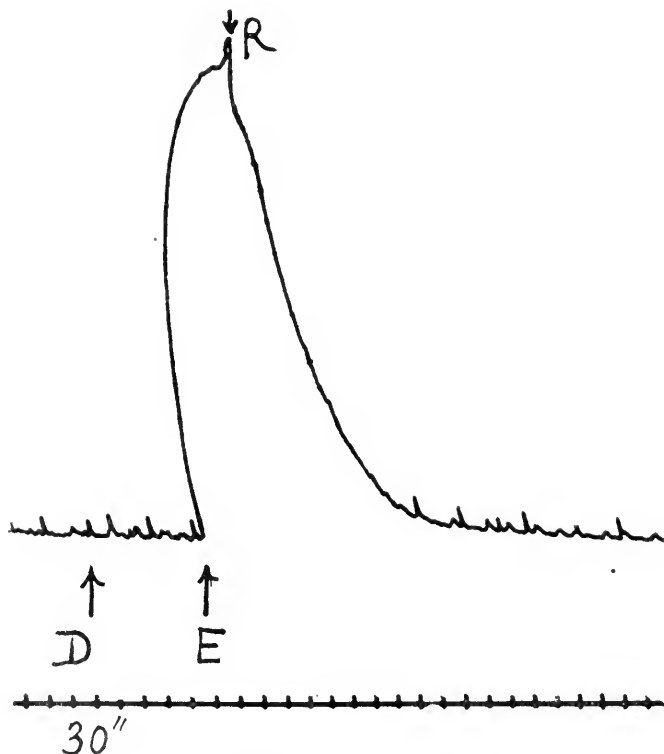


FIG. 22. Continuation of figure 21. After soaking for three hours in 10 per cent normal guinea-pig serum. *D*, 0.1 cc. horse serum. *E*, 0.1 mg.  $\beta$ -imino-zylolethylamine.

removed, was placed in a Buchner funnel, and the fluid, as it flowed from the vena cava, was collected in a beaker placed below the funnel and returned at intervals to the Marriotte bottle. In this way perfusion for an indefinite period could be carried out with 100 cc. of serum, obtained from twenty sensitive guinea-pigs, and diluted to 500 cc. with Ringer solution. The guinea-

pig was killed and bled out as usual. The abdomen was opened and the intestines removed and one horn of the uterus was then tied off, excised, and suspended in the testing bath, to make it certain that the guinea-pig was originally normal, and possessed no accidental sensitiveness to horse serum. The animal was then transected and the cannula tied into the aorta as low down as possible. The vessels were first washed through with Ringer's solution and the cannula then connected to the perfusion apparatus. The head of pressure, from the surface of fluid in the oxygenation tower to the aortic cannula, was about 90 cm. of

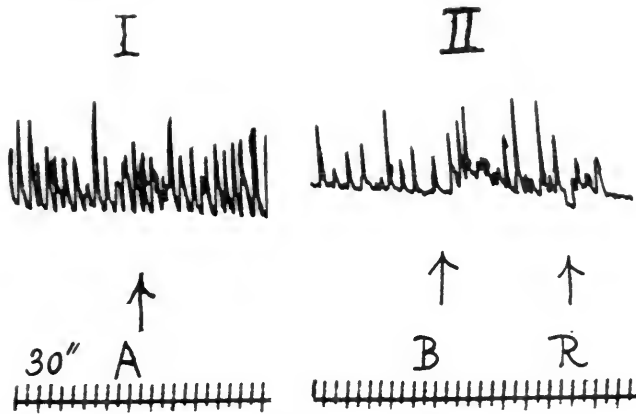


FIG. 23. Normal uterus. Bath volume 250 cc. I, control horn not perfused. A, 0.5 cc. horse serum. II, second horn after perfusion for one hour with 20 per cent sensitive serum, and washing out. B, 0.5 cc. horse serum.

water. Under this pressure perfusion proceeded at a rate which did not diminish perceptibly in five hours, when diluted serum was used; whereas, when perfusion is carried out with pure Ringer's solution, it begins to get slower in an hour, owing to the resistance caused by oedema of the tissues. The uterus was covered with a small swab of cotton wool, which was kept moist and warm by inhibition of fluid escaping from the cut vena cava. At the end of the period a second perfusion with 500 cc. of Ringer's solution was given before testing.

Figures 23 and 24 illustrate the effects of such a perfusion with 20 per cent horse-sensitive serum carried out for one hour

and five hours, respectively. In figure 23 it will be seen that the uterus, of which the control horn gave no trace of response to 1 in 500 horse serum at *A*, showed, after one hour's perfusion, at most a doubtful trace of response to the same dose. The effect is one which, by itself, is negligible. Another uterus, how-

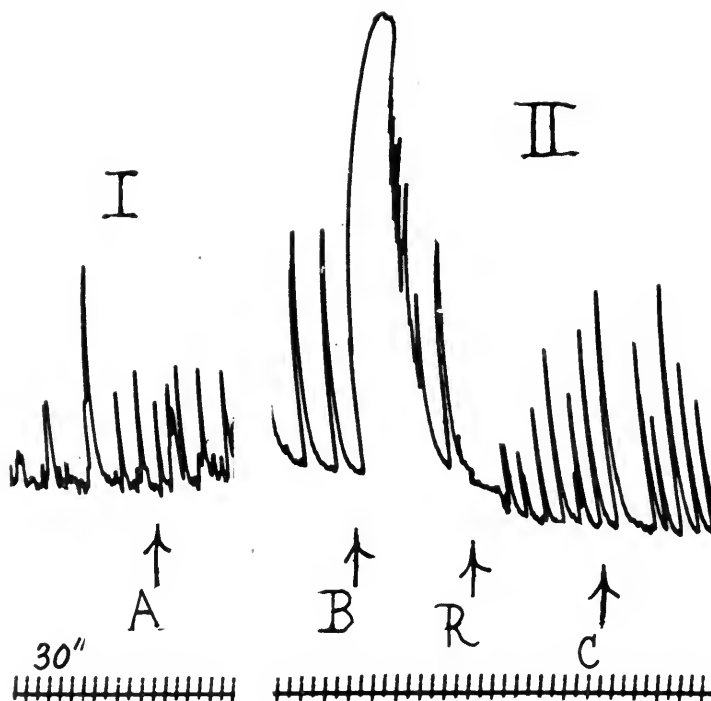


FIG. 24. Normal uterus. Bath volume 250 cc. I, control horn, not perfused. *A*, 0.5 cc. horse serum. II, second horn, after five hours perfusion with 20 per cent sensitive serum, and a final perfusion with 500 cc. Ringer's solution. *B*, 0.5 cc. horse serum. *C*, the same.

ever, which was similarly perfused for five hours, gave the very definite response to horse serum illustrated in figure 24. The uterus had a larger automatic rhythm than the other, and this became even larger as the result of the five hours' treatment; but it was initially as completely insensitive to horse serum, and the effect shown in figure 24 is quite definite, and widely outside



the limits of its automatic activity. It must be remembered that, even with intravenous injection, passive sensitiveness in the whole animal is only just becoming perceptible five hours after injection. The result with the perfused organ is, therefore, exactly what might be expected, on the assumption that the process of passive sensitisation in the guinea-pig consists of the fixation of antibody by the plain muscle cells.

#### 8. THE INCUBATION PERIOD

It seemed of interest to compare the sensitiveness of the plain muscle at different periods after the sensitising injection. The earlier accounts of anaphylaxis in the guinea-pig indicated ten days as the period which must elapse before sensitiveness could be detected. Later workers, using intravenous injection for the second dose, observed symptoms as early as the sixth day: while Friedberger and Mita<sup>37</sup> observed a small rise of temperature, which they regard as an index of a very low grade of sensitisation, on reinjection as early as the day after the sensitising dose. It seemed probable that the appearance of sensitiveness in the plain muscle would correspond rather with the earliest period at which obvious signs of distress occur on reinjection, and this proved to be the case.

A series of young guinea-pigs of approximately equal weight were injected, at intervals of two days, with 0.1 cc. of normal horse serum in each case. They were finally tested all on the same day. The uteri were suspended without perfusion in this case, as previous experience had shown that this procedure did not materially affect the result. The bath-volume was 250 cc. and the temperature was carefully kept at 38°C. throughout the experiment. The material used for testing was normal horse serum, fifteen days old, a supply of the purified product not being available on the day when the test had to be made. No dose larger than 0.5 cc. of serum (1 in 500) was therefore used, and it may be that the earliest traces of sensitiveness were missed through insufficient dosage. It seemed more important, how-

<sup>37</sup> Zeitschr. f. Immunit., x, p. 216, 1911.

ever, to keep the dose well below the limit of normal toxic action, and the results obtained, and illustrated in figure 25, correspond well with those seen by injecting the animal. Briefly they were as follows. Up to and including the sixth day there was no sign of response to the highest dose employed (0.5 cc.). On the eighth day, one horn gave a trifling and doubtful response to 0.1 cc. of serum (1 in 2500), the other horn a definite, but submaximal and quickly evanescent response to 0.5 cc. (1 in 500). On the tenth day the response to 0.1 cc. was much larger than that to 0.5 cc. on the eighth day, but was not quite maximal. The maxima of which these uteri were capable are indicated by a horizontal line above the contraction curve. This was traced by rotating the drum by hand, with the uterus contracted to its minimum length in response to 0.1 mg. of  $\beta$ -Iminazolyethylamine.

On the twelfth and fourteenth days maximal contractions were given in response to 0.1 cc. of horse serum and would doubtless have been obtainable with even smaller doses. The first appearance of plain muscle sensitiveness in this series occurs, therefore, somewhere between the sixth and eighth days after the sensitising injection, and rapidly increases up to the twelfth day. It will be seen, incidentally, that no great delicacy can be claimed for the method in the direction of recognising minute degrees of sensitisation; its delicacy lies in another direction, namely, in the minuteness of the dose of antigen with which an obvious response is obtained when sensitiveness is fully developed.

#### 9. THE PART PLAYED BY SENSITISATION OF THE PLAIN MUSCLE IN ANAPHYLACTIC SHOCK, AS SEEN IN THE GUINEA-PIG

There are some features in the anaphylactic shock of the guinea-pig, especially in its milder forms, which cannot be related to the effect on plain muscle. The signs of cutaneous irritation for example presumably depend on action of another kind, except in so far as they are secondary to the raising of the hair, which may possibly be due to sensitiveness of the pilomotor muscles. The haemorrhagic condition of the intestines, indicating a poison-

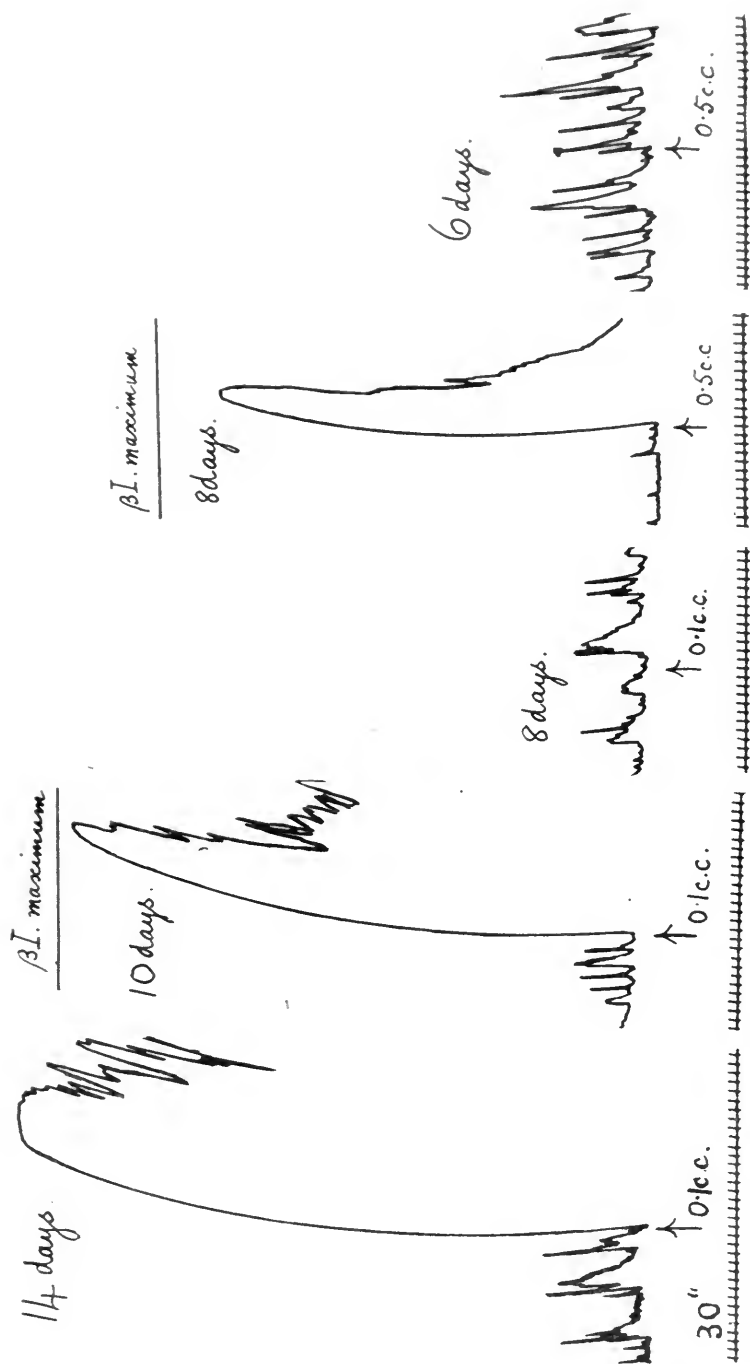


FIG. 25. Uteri from guinea-pigs sensitised with 0.1 cc. of horse serum hypodermically, with varying incubation periods, as indicated. Bath volume 250 cc. Volume indications attached to arrow signals indicate test doses of horse serum.

ing of the capillary endothelium, and the remarkable fall of temperature (Pfeiffer<sup>38</sup>), due to a diminished general metabolism, as shown by the respiratory quotient (Loening<sup>39</sup>), are other effects which indicate that excitation of plain muscle tonus represents only one aspect of a complex action. These other effects may be due to sensitisation of cells of another type, but on that point we have no direct evidence. In the preceding sections I have given evidence of the very high sensitiveness of the washed plain muscle to the specific antigen. It remains to be discussed whether this is the sole cause of the plain muscle reaction as seen in the entire animal. It is obvious at once that a sensitiveness of the order exhibited by the isolated uterus would quite adequately account for the characteristic asphyxial death, if it were exhibited by the bronchiolar muscle *in situ*. This cannot, however, be assumed to be the case without further examination. We have seen that the isolated uterus even of a normal guinea-pig will give, to large doses of fresh guinea-pig serum, a reaction of the same type as the anaphylactic uterus gives to very small doses of the specific antigen. Yet the effect of injecting fresh guinea-pig serum into the normal animal in considerable doses is not always or even usually fatal. We must believe, therefore, that the body has some means of dealing with the toxic constituent of normal serum, so as to prevent its producing the full effect obtained with the isolated plain muscle, or that the latter is in a condition of abnormal sensitiveness to the kind of stimulus with which we are dealing. Again, it has been shown, by Manwaring,<sup>40</sup> and confirmed by Voegtlin and Berthelm,<sup>41</sup> whose method escapes the criticism which might have applied to Manwaring's, that the anaphylactic dog shows no reaction to horse serum when the liver is excluded from the circulation. So that the assumption, that the bronchiolar spasm, and other plain muscle effects in the guinea-pig, are solely dependent on sensitisation of the muscle fibers themselves, neces-

<sup>38</sup> Wien. klin. Wochenschr., xxii p. 14, 1909.

<sup>39</sup> Arch. f. exp. Path. u. Pharm., lxvi, p. 84, 1911.

<sup>40</sup> Zeitschr. f. Immunit., viii, p. 1, 1910.

<sup>41</sup> Journ. of Pharm. and Exp. Therap., ii, p. 507, 1911.

sitates the conclusion that the mechanisms of the shock in the dog and the guinea-pig are entirely different with regard to this characteristic feature. The question of participation of the liver in the anaphylactic shock of the guinea-pig was easily tested. In order to avoid the modification of the bronchiole reaction by anaesthetics the animal was first etherised and a tracheal cannula inserted, and then the brain was destroyed by a probe pushed through the *foramen magnum*. Artificial respiration with pure warmed air was then applied. The abdomen being opened, I ligatured successively the inferior mesenteric artery, the coeliac axis at its origin from the aorta, and the portal vein. The rectum was double-ligatured and cut across and the oesophagus ligatured tightly just below the diaphragm. The liver and stomach and intestine were thus completely excluded from circulation. The chest was then opened so that the rhythmic inflation and expiratory collapse of the lungs could be watched. A cannula was tied into the jugular vein for injections. The experiments were made on sensitive survivors from antitoxin (horse serum) standardisations and on normal controls. In the latter the injection of 1 cc. of either horse or sheep serum (a few days old) produced an acceleration and augmentation of the heart-beat, but in no way disturbed the rhythmic inflation and collapse of the lungs. In the guinea-pigs sensitised to horse serum injection of 1 cc. of sheep serum was similarly ineffective, but 0.1 cc. of horse serum, after the brief interval necessary for its carriage to the lungs in the circulation, produced a rapid diminution of pulmonary ventilation, which, in a further few seconds, progressed to complete immobilisation. When once the block had become complete no increase of the stroke of the pump, even with complete closure of the lateral opening of the tracheal cannula, could force air into the lungs, which remained permanently collapsed. If the ventilation were increased at the first sign of obstruction, the lungs became distended to the maximum and remained permanently in this condition. The experiment was varied by excluding the lower half of the animal from circulation, the aorta and vena cava being tied just above the diaphragm, but the result was the same.

In some of the earlier investigations on anaphylaxis evidence was obtained which seemed to point to the brain as the primary seat of the reaction to reinjection. This evidence had largely lost its validity, but it seemed desirable to exclude this possibility also. In another set of horse-sensitive guinea-pigs, therefore, the liver, stomach and intestines were excluded from circulation as above. The chest was then opened, the pericardium slit up, and the innominate and left subelavian arteries ligatured close to the arch of the aorta. The brain was pithed and the anaesthetic turned off. The typical immobilisation of the lungs again resulted from injecting 0.1 cc. horse serum into the jugular vein.

Finally, in another series, the pulmonary circulation was replaced by perfusion with pure Ringer's solution. A tracheal cannula was first inserted and artificial respiration established under chloroform and ether. The front wall of the chest was cut away, the pericardium opened, and a ligature passed round the pulmonary artery. Then the ventricles were cut away, and the blood, as it escaped, washed away with warm Ringer's solution. A cannula filled with the same was tied into the pulmonary artery. This was connected to a small reservoir of Ringer's solution, holding about 20 cc. and immersed in a constant-temperature bath at 40°C., similar to that used for the isolated organ. The level in the reservoir was maintained constant at about 30 cm. above the pulmonary artery by means of a tube connected with a Marriotte bottle. The anaesthetic was now turned off, respiration being continued with pure air, and the perfusion begun. The left auricle was slit open, and clots removed if necessary, to allow free egress for the Ringer solution returning by the pulmonary veins. When all the blood had been washed away, so that the lungs appeared pure white, and apparently pure Ringer's solution flowed from the left auricle, the dose of warmed serum was added to the reservoir. In the case of normal guinea-pigs 1 cc. of horse or sheep serum produced no effect, or at most a doubtful trace. The same was true with the sensitive animal when 1 cc. of sheep serum was given. In the latter case, however, 0.1 cc. of horse serum; after the interval necessary

for it to reach the lung circulation, produced the usual immobilisation. In some experiments the lungs and trachea, after the perfusion had been started, were removed from the chest and fixed to a board, where the rest of the experiment was performed: this did not affect the result in any way. There seems no necessity, therefore, for assuming any other than the immediate action of the antigen on the sensitised muscle, in explanation of that feature of the anaphylactic shock, as seen in the guinea-pig, which determines its acutely fatal issue. On the other hand it is possible, and even probable that those features in which it resembles the effect in the dog, and which only become prominent when the effect is not rapidly fatal, such as the loss of coagulability of the blood, necessitate the integrity of the liver circulation. How far the effects on the bowels and bladder are primary muscular effects, how far secondary to asphyxia, is a point which I have not yet examined.

#### 10. A SUGGESTED MEDICO-LEGAL APPLICATION

There are two possible ways in which the technique described in previous sections could be used for the specific identification of blood, etc., for medico-legal or other purposes.

1. As in methods already current, the suspected material might be used for sensitising a guinea-pig. After allowing sufficient time for full sensitisation, the uterus could be excised and a horn suspended. Sera from the various species possibly implicated could be added in small doses until the one was found which caused the typical response (fig. 26). The other horn, kept meanwhile in warm oxygenated Ringer, could then be used for determining the lower limit of dosage of the serum which caused a response.

2. Guinea-pigs could be sensitised with a small injection of known serum from the suspected species, e.g., human serum. After the usual incubation period one pig would be killed, the uterus excised, and the degree of sensitiveness of the first horn to human serum tested. If the sensitiveness were not of a high order, another pig could be tried at once, or after a few days

longer incubation period. When a uterus was found which gave a large and clear response to, say, 1 in 100,000 human serum, the other horn, kept meanwhile in warm oxygenated Ringer, could be suspended in a small volume such as 10 cc. of Ringer's

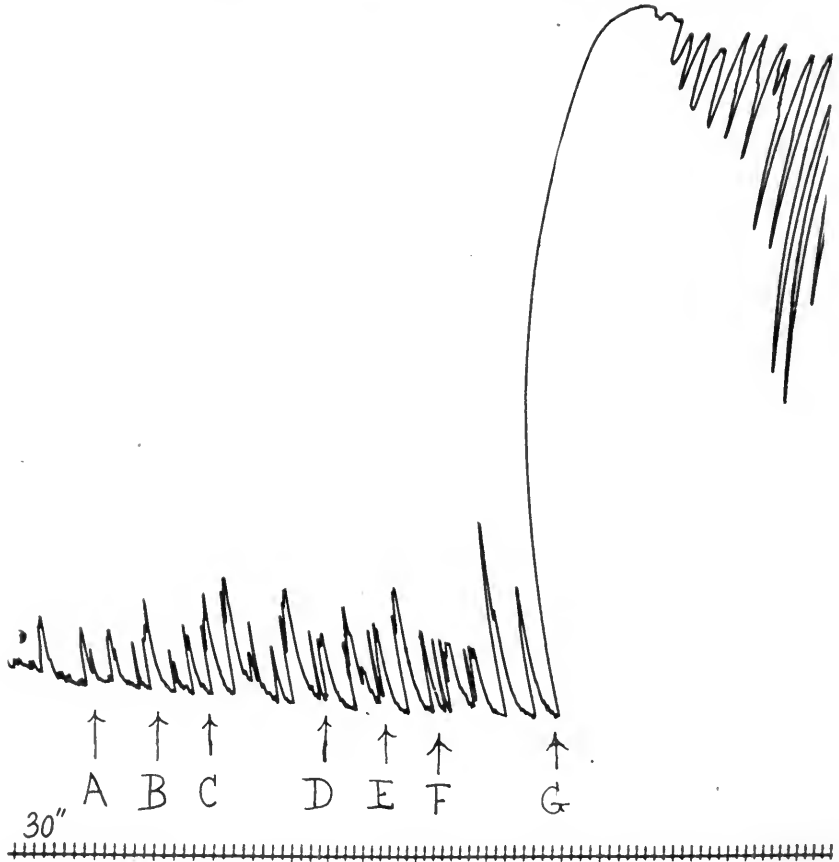


FIG. 26. Sensitisation: 1/400 cc. antitoxin (horse) + 1 test dose of toxin, fourteen days. Doses in each case 0.1 cc. A, sheep. B, cat. C, rabbit. D, dog. E, human serum. F, egg-white. G, horse serum.

solution. A dose of the suspected material could then be added, and if no reaction were produced it would be clear that the dose contained less than 1/10,000 cc. of human serum, which should be sufficient evidence, in any ordinary case, that the blood under



examination was not human. If, on the other hand, a decided response were produced, it would only be necessary further to test the action of the specimen on a normal uterus, so as to exclude primary non-specific toxicity. The specificity of the sensitisation could be controlled by giving doses of other sera before the suspected sample. Figure 26 shows what a clear specific indication can be thus obtained. A uterus from a guinea-pig sensitised to horse serum is tested with sheep, cat, rabbit, dog and human serum, and egg-white. The automatic rhythm gradually increases, as it frequently does when nothing is added; but no definite response of any kind is seen till 0.1 cc. of horse serum is added. This second method of applying the reaction has the advantage that the specific sensitiveness of the organ can be definitely verified before applying the decisive test; whereas the failure of response, when the suspected material is given as a sensitising injection, may leave room for doubt as to whether the guinea-pig has exceptionally escaped sensitisation. It will be seen, further, that the dose of the specific antigen needed to produce a response, with a uterus of high sensitiveness and a bath of small volume, is no greater than that which is necessary for producing sensitisation with a reasonable degree of certainty.

## 11. SUMMARY AND DISCUSSIONS

The conclusions from the foregoing results may be summarised as follows:

1. Plain muscle from an anaphylactic guinea-pig, freed from all traces of blood and serum, has a very high degree of sensitiveness to the specific sensitising protein. The plain muscle of the virgin uterus is especially suited to the demonstration of the condition, and exhibits a definite rise of tonus in response to extreme dilutions of the antigen.

2. The effect is practically immediate, i.e., the delay is not obviously more than can be attributed to the method of application of the antigen.

3. The response is not a mere exaggeration of the reaction which normal plain muscle gives to fresh sera in general. Preparations

of purified protein can be obtained (e.g., serum globulin precipitated by Gibson's method, or egg-albumen crystallised by Hopkins' method), which have no effect on the normal plain muscle, but are as toxic for the anaphylactic plain muscle as the native proteins.

4. One dose of the specific antigen, in sufficient concentration to produce "maximal" response of the anaphylactic plain muscle, completely desensitises the latter to further doses of any dimensions, provided that the experiment is not complicated by the use of an antigen preparation of normal toxicity. Either normal or anaphylactic plain muscle gives repeated responses to successive large doses of a normally toxic serum or other native protein, but this phenomenon is not anaphylactic response.

5. When sensitising doses of several antigens are given, a multi-sensitisation of the plain muscle can be demonstrated. Desensitisation of the muscle to one antigen is not without effect on its sensitiveness to the others.

6. The washed plain muscle from guinea-pigs immunised to an antigen, by a series of injections, is sensitive to the antigen, like that from anaphylactic pigs. But the sensitiveness is, in this case, less rigidly specific, e.g., plain muscle from a guinea-pig immunised to horse serum showed a subsidiary sensitiveness to sheep-serum.

7. The sensitiveness of the washed plain muscle is seen in passive as in active anaphylaxis, whether the serum producing passive sensitisation is obtained from sensitive or from immune guinea-pigs.

8. The actively or passively sensitised plain muscle, after being desensitised *in vitro*, can be resensitised *in vitro* by mere contact for some hours with a not too great dilution of sensitive serum. It has not yet been found possible to sensitise normal plain muscle in exactly the same way; but perfusion of a normal uterus for five hours, with diluted serum from sensitive guinea-pigs, produced a decided passive sensitisation.

9. The response to the specific antigen of the bronchioles of the anaphylactic guinea-pig is not impaired by excluding the abdominal viscera and the brain from circulation, and is pro-

duced with apparently undiminished vigour in the isolated lungs perfused with Ringer's solution.

10. It is suggested that the method here described may find medico-legal application.

Conclusions 1 and 5 are confirmatory of those of Schultz, though differing in some details, particularly as to the very high degree of sensitisation which my experiments demonstrate. Conclusions 2 and 3 are at variance with Schultz's conclusions, and I have indicated the difference in our methods on which this difference of conception depends. Schultz regards the toxic action of fresh sera as an effect of foreign proteins as such, and the anaphylactic reaction as an exaggeration of this effect in the case of one foreign protein. I regard the normal toxic effect of these preparations as an adventitious action due to unknown constituents, which has a deceptive similarity to the effect of a pure protein on muscle specifically sensitised to it, and which for that reason must be carefully excluded, before the pure anaphylactic effect can stand out in efficient contrast. The correctness of this latter conception seems to me sufficiently proved by the correspondence, in my experiments, between anaphylactic effect on isolated muscle, and the same effect in the whole animal, especially in regard to the process of desensitisation (anti-anaphylaxis), and to the complete contrast between the anaphylactic and the normal condition as regards reaction to the sensitising antigen. I have mentioned in an earlier section that in one experiment Schultz used a preparation which I should regard as ideal for the purpose, viz., egg-albumen crystallised by Hopkins' method. He tested the action of this on a normal guinea-pig's intestine and found that "the muscle either relaxes or does not contract." He attributes this "to the influence of the acid retained by the protein during the crystallising stage, though none could be detected by litmus; or possibly to the solution being too dilute." Schultz was, in fact, using in this case a protein purified from natural toxic concomitants, and he would have found it an admirable stimulant of plain muscle from a guinea-pig sensitised to egg-white, if he had tried the experiment. In another respect my results agree with those of Schultz, namely,

in demonstrating that isolated plain muscle from an *immune* guinea-pig may be very sensitive to the immunising antigen. This emphasises the necessity of distinguishing clearly between the condition of "anti-anaphylaxis" on the one hand, which, if the isolated organ gives a correct picture of the process, must be attributed to actual desensitisation of the plain muscle, and the "immunity," on the other hand, which results from a series of injections of the antigen. This immunity can only be attributed to the presence of a circulating antibody, which destroys or saturates the antigen, before this can reach the sensitive plain-muscle cells.

So far the experiments merely demonstrate directly the correctness of the views as to the relation between antibody, antigen, and sensitive tissue, current in the writings of a number of observers, and hitherto founded on deductions from observations on the entire animal. The same is true of the direct demonstration of the fixation of antibody to muscle cells in the process of passive sensitisation. The previous indirect evidence on these points is well summarised by Anderson and Frost.<sup>42</sup>

As to the identity with precipitin, or separate nature of the anaphylactic antibody, my results provide no direct evidence. They appear to me, however, to give an indication as to the probable nature of the anaphylactic reaction which is worth some consideration.

At first sight the theory ascribing the symptoms of anaphylactic shock to the production of a poison by parenteral digestion has an attractive plausibility. Nor is it by any means excluded by the demonstration of the specific response in tissue freed from blood, since, given the demonstrated attachment of antibody to muscle-cells, or its incorporation into them, a poison produced by the interaction between antigen and an antibody so situated, would arise under conditions ideal for the exhibition of its activity. But any theory involving the production of a toxic cleavage product by ferment action encounters a very serious difficulty in the time-relations of the effect. The specific

<sup>42</sup> Hyg. Lab. Bulletins, No. 64, Washington, 1910.

antigen acts on the isolated plain muscle with as little delay as a direct stimulant, such as pilocarpine,  $\beta$ -Iminazolyethylamine, or pituitary extract; that is to say, the apparent delay is almost all accounted for by the mechanical imperfection of the method of applying the drug, or in this case the antigen, to the muscle.

Apart from the absence of a coarse latent period, the rest of the time-relations of the effect are very difficult to harmonise with any form of the ferment theory. On such a theory, one would expect a gradual onset and slow rise to a maximum. But the onset is sudden, and the rate of progress to the maximum, when sensitiveness is fully developed and the dosage not too minute, is apparently limited only by the contraction rate of the plain muscle. After a few minutes at the maximum the effect begins to decline, and the muscle is then insensitive to further doses. In the form of the contraction curve, as in the brief latency, the effect recalls that of a powerful stimulant drug, added directly to the bath.

Such considerations do not, of course, at all affect the validity of the evidence that serum from a sensitive animal, or, still better, from an immune animal, when incubated with the corresponding antigen, exhibits a power of specific proteolysis. Whether the antibody be precipitin or not, the antigen-antibody complex will probably be attacked by the proteolytic complement; it is not surprising that the result of its action, as of partial peptic digestion, or other forms of gentle hydrolysis, should be the production of substances having an action of similar type to that which we are discussing. A good deal of the significance which such experiments appeared to possess has recently been greatly weakened by the experiments of Ritz and Sachs<sup>43</sup> and of Doerr and Pick,<sup>44</sup> who showed that a toxicity, similar to that acquired by digesting with specific antigen or precipitum, could be imparted to normal guinea-pig serum by digesting it with kaolin or kieselguhr. Similarly Doerr and Moldovan,<sup>45</sup> by showing that many of the features of the anaphylactic shock can be reproduced by intravenous

<sup>43</sup> Zentralbl. f. Bact. (Ref.), l, 1911; Tag. d. Frei. Verein f. Mikobiol., p. 48\*.

<sup>44</sup> Wien, klin. Wochenschr., xxv, p. 337, 1912.

<sup>45</sup> Biochem. Zeitschr., xli, p. 27, 1912.

injection of inorganic colloidal solutions, such as colloidal silica, have greatly weakened the supposed significance of the parallel action of certain protein cleavage products. This observation has the further value of suggesting the direction in which an explanation may be sought, for the action of the specific antigen on the sensitised animal. These inorganic colloids have the property of causing a mutual precipitation when they are mixed with a solution of protein. Doerr and Moldovan suppose that the effect of the antigen, in contact with the antibody of the sensitised animal, is similarly to alter the state of aggregation of the colloids in the blood-plasma, and induce changes of surface tension, which are responsible for the effect on the plain muscle and other tissues. In the light of the evidence produced by Schultz and by myself, this view, so far as the guinea-pig's plain muscle is concerned, needs modification, to the extent of transferring the reaction between antibody and antigen to the plain-muscle fiber itself. A good deal of evidence is available in favour of the view put forward by Friedberger,<sup>46</sup> and supported by Doerr and Russ,<sup>47</sup> that precipitin and anaphylactic antibody ("toxogenine," "anaphylactin," "sensibilisine" or "allergin") are identical. But there is no necessity for supposing that the process taking place in the plain-muscle fiber, and resulting in the characteristic rapid rise of tonus, is an actual precipitation. The effect can probably be accounted for by the mere initiation of those changes in the state of aggregation of the colloidal particles, which, when antibody and antigen are present in appropriate proportions, and sufficient time is allowed, result in the formation of a visible precipitate. It is not, however, necessary to assume the identity of precipitin and anaphylactic antibody; if they be identical, as seems very probable, it is not necessary that the antibody should be present in such proportion as to give with the antigen a visible precipitate. All that is needed is that the antibody should have such a specific physical relation to the antigen that, when the two meet, a disturbance of the conditions of colloidal solution is set up in the muscle fiber. The

<sup>46</sup> *Zeitschr. f. Immunit.*, ii, p. 208, 1909.

<sup>47</sup> *Ibid.*, iii, p. 706, 1909.

suggestion has an obvious relation to such a conception of muscular contraction as that put forward by Lillie.<sup>48</sup> It is hardly necessary to point out how well such a conception fits the phenomena recorded in the previous sections. The action of the antigen in extreme dilutions, the saturation of the antibody (desensitisation), the cessation of the effect when the union of antibody and antigen may be supposed to be complete; all find their reasonable explanation.

What then is the significance of the striking parallelism between the action of certain protein cleavage-products, and the symptoms of the anaphylactic reaction? The parenteral digestion theory offers these as substances of known action, the production of which would explain the unknown action of normally inert proteins on the sensitive animal. It may be suggested that the position is the reverse of this; that the action of the antigen on the sensitised muscle is, in reality, the one case in which we have as yet any indication of the possible nature of the effect produced by poisons of this class. One group of substances having this action, the protamines, have the property of forming precipitates with the soluble proteins (Kossel)<sup>49</sup> and the fact may be of significance in this connexion. It may be suggested that a further study of the nature of the physical changes, which follow the mixture of serum or muscle plasma, from sensitised or immunised animals, with the corresponding antigen, may throw light not only on the action of antigen on sensitised tissues, but on the similar action of the intermediate products of protein hydrolysis, and even on that of so simple a substance as  $\beta$ -Iminazolyethylamine.

It has been previously emphasised, that it is only to the guinea-pig's plain muscle that my conclusions can be directly and safely applied. A word is desirable with regard to the anaphylactic response as seen in other species. There seems evidence of an analogous tissue sensitisation in the rabbit, but in this case it has hitherto been demonstrated in the cardiac muscle only (Cesaris-Demel).<sup>50</sup> Evidence is wanting as to the plain muscle response

<sup>48</sup> Amer. Journ. of Physiol., xvi, p. 117, 1906; xxi, p. 200, 1908.

<sup>49</sup> Zeitsch. f. Physiol. Chem., xxii, p. 186, 1896.

<sup>50</sup> Arch. Ital. de Biol., liv, p. 141, 1910.

of this species, but it may be noted that, according to the observations of Arthus,<sup>51</sup> and of Auer,<sup>52</sup> the cardiac effect would appear to be as predominant in the rabbit's anaphylactic reaction as the plain muscle effect is in that of the guinea-pig. In the dog, Manwaring, and Voegtlin and Bertheim, have clearly demonstrated that the primary effect is in the liver. In a few experiments of my own on the anaesthetised dog, the same point was perfectly clear; with the liver excluded from circulation reinjection caused no trace of shock, but on release of the clamp on the hepatic artery it at once developed. In the cat Schultz<sup>53</sup> claims to have demonstrated a plain muscle sensitisation similar to that seen in the guinea-pig, but he was apparently working with fresh sera, of such high primary toxicity as to cause fatal effects in a proportion of normal cats, when injected in moderate dosage; so that his conclusions are vitiated by the confusion which, as I have suggested, complicates his results on the guinea-pig. In the dog I have as yet detected no trace of plain-muscle sensitisation, and all the evidence would appear to point to the liver cells as the seat of the primary reaction; and it may well be that they are sensitised by the presence of antibody as the plain-muscle cells are in the guinea-pig. How far the circulatory effects in the dog are due to mechanical obstruction in the liver capillaries, how far to the liberation into the blood of toxic products from poisoned liver cells, is a point that appears to need further investigation. The whole process is so much slower in the dog that such secondary intoxication cannot be excluded, and it must presumably be at least the main factor in the production of symptoms when the liver is supplied by the hepatic artery only, and the portal circulation cut off or diverted. Nor can it be assumed that such liver-sensitisation is absent in the guinea-pig; it seems more probable that the sensitisation of the plain muscle in this species is an additional factor, which produces, in many cases, an effect so promptly fatal, that the more general type of reaction, as seen in the dog, has not time to develop. But

<sup>51</sup> C. R. Soc. de Biol., lv, p. 817, 1903.

<sup>52</sup> Journ. of Exp. Med., xiv, p. 476, 1911.

<sup>53</sup> Journ. Pharm. and Exp. Therap., iii, p. 299, 1912.



this is another point on which further evidence is needed; and such evidence may be expected to give indications as to the place of origin of the antibody, the attachment of which to plain or cardiac muscle fibers, on the one hand, or accumulation mainly in the liver-cells, on the other hand, would appear to be the determining factor in the different types of anaphylactic shock exhibited by those species, in which the phenomenon has hitherto been subjected to accurate physiological analysis. Such further examination, of the relation of effects seen in the guinea-pig to those seen in other species, I hope to undertake in the immediate future.



## THE CONGENITAL TOLERANCE OF THE RAT TO STROPHANTHUS

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Many investigations have been undertaken to determine the cause of the congenital tolerance which is displayed by certain species of animals to certain drugs, and those investigations tend to show that congenital tolerance to drugs is not always of the same nature but may be due to one, or to a combination, of several possible factors.

It is well known that, in proportion to its body weight, the rat exhibits a much greater resistance than the rabbit to the toxic action of digitalis glucosides and to allied substances which have a similar pharmacological action. This investigation was undertaken to determine to what extent, if any, this tolerance of the rat to the action of those glucosides is due to a relative absence in this animal, of suitable receptors upon which they can act. Little evidence, and less that is unequivocal, has been adduced in favor of the existence of this particular variety of congenital tolerance to drugs.

The experiments were first begun with digitalin and digitalein but it was found almost impossible to kill a rat with them, and recourse was then had to strophanthus.

An extract of strophanthus was prepared in sufficient quantity to perform all the necessary experiments. It was made in the way recommended by Fraser, namely, by removing the oil from the powdered seeds by extraction with ether, and then preparing a dry alcoholic extract from the residual powder. The extract was freshly dissolved for each experiment.

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A. MINIMUM-LETHAL DOSE BY SUBCUTANEOUS INJECTION OF  
STROPHANTHUS EXTRACT

TABLE I  
*Rabbits*

EXPERIMENT	WEIGHT OF RABBIT	DOSE PER KILO	ACTUAL DOSE	RESULT
	<i>grams</i>	<i>grams</i>	<i>grams</i>	
1	2400	0.0005	0.0012	Recovery
2	1800	0.0008	0.0014	Recovery
3	2100	0.001	0.0021	Death in 2 hours
4	2300	0.0015	0.0034	Death in 48 minutes

TABLE II  
*Rats*

EXPERIMENT	WEIGHT OF RAT	DOSE PER KILO	ACTUAL DOSE	RESULT
	<i>grams</i>	<i>grams</i>	<i>grams</i>	
5	210	0.01	0.0021	Recovery
6	170	0.015	0.0025	Recovery
7	170	0.02	0.0034	Recovery
8	170	0.025	0.0043	Recovery
9	170	0.03	0.005	Death in 3¼ hours
10	200	0.04	0.008	Death in 2½ hours

The minimum lethal dose of this extract of strophanthus by subcutaneous injection is, therefore, for the rabbit 0.001 gram per kilogram.

The minimum lethal dose for the rat is, therefore, 0.03 gram per kilogram, or thirty times greater than the minimum lethal dose per kilogram for the rabbit.

The question arises—what is the cause of this tolerance or partial immunity?

Strophanthus kills chiefly, if not entirely, by its toxic action on the heart. The fact that a larger dose (per kilo) is required to kill the rat than suffices to kill the rabbit may be due to several possible causes, which may eventually be reduced to two: (1) Either the strophanthus, when subcutaneously injected, reaches the heart of the rat in less concentration than it reaches the rabbit's heart (from inferior absorption, superior excretion, or increased destruction in the body); or (2) Strophanthus reaches

the heart in the case of both animals in the same (active) concentration, but the rat's heart is less susceptible to its action than the rabbit's heart. Only the second factor (2) has been investigated because it has been found sufficient by itself to explain the congenital tolerance of the rat to strophanthus.

#### B. ACTION ON THE ISOLATED HEART

The isolated hearts of rabbits and rats were perfused with various concentrations of strophanthus, Brodie's apparatus being used. The perfusion medium employed was Ringer's solution in which the strophanthus also was dissolved. The temperature of the fluid during the experiments was kept between 36° and 38°C.

The results of those experiments are given in the following tables:

TABLE III  
*Isolated rabbit's heart*

EXPERIMENT	STRENGTH OF SOLUTION	RESULT
11	1 in 20,000,000	Reduction of diastolic relaxation (but not arrest) in 1 hour
12	1 in 10,000,000	Arrest in systole in 40 minutes (v. fig. 1)
13	1 in 5,000,000	Arrest in systole in 34 minutes
14	1 in 500,000	Arrest in systole in 9 minutes
15	1 in 50,000	Arrest in systole in 2½ minutes

TABLE IV  
*Isolated rat's heart*

EXPERIMENT	STRENGTH OF SOLUTION	RESULT
16	1 in 1,000,000	Slight reduction of rate and amplitude in 1½ hours
17	1 in 500,000	Reduction of diastolic relaxation (but not arrest) in 1 hour (v. fig. 2)
18	1 in 250,000	Systolic arrest in 9 minutes
19	1 in 250,000	Systolic arrest in 8 minutes

It is obvious from these results that the rat's heart is much less susceptible than the rabbit's heart to the toxic action of strophanthus. Such experiments on the isolated heart get rid of

complications which may arise from any possible antagonistic action of the rat's serum, because the heart is perfused with saline solution for at least fifteen minutes before the strophanthus solution is turned on, and in that time all but the merest traces of blood is removed from the heart. One is dealing, therefore, with a comparatively simple reaction between strophanthus and heart muscle.

It has already been shown that, subcutaneously injected, a dose thirty times larger is required to kill the rat than suffices to kill the rabbit.

If the tolerance shown by the rat is due only to an insusceptibility of the heart and not to other factors, then it should be found that the isolated heart of the rat should require somewhere about thirty times as strong a solution to arrest it as is sufficient to arrest the isolated heart of the rabbit. For various reasons, into which it is not necessary to enter here, it is not to be expected that this ratio will be found exact. In order, however, to institute a comparison it is more important to compare the effects of more dilute solutions which arrest the isolated heart at a time approximating that which the subcutaneous minimum lethal dose requires to kill the intact animal. Though the minimum lethal dose takes at least two hours to arrest the heart, it was not thought advisable to adopt this time for the arrest of the isolated heart by perfusion, because there is a possibility that the isolated heart may in some cases cease to beat in that time without the action of strophanthus. This disability, however, does not arise in perfusion for one hour.

If this latter time-standard, to some extent arbitrary, be adopted, then it is apparent from Tables III and IV that a solution of 1 in 500,000 has no more toxic effect on the rat's heart than a solution of 1 in 20,000,000 has on the rabbit's heart, i.e., than a solution forty times as strong: it has less action than a solution of 1 in 10,000,000 has on the rabbit's heart, i.e., than a solution twenty times as strong (v. figs. 1 and 2). The ratio of toxicity for the isolated heart is therefore: rabbit : rat = 1 : 20 to 40. This accords so nearly with the ratio of the minimum lethal dose, viz., rabbit : rat = 1 : 30, as to warrant the con-

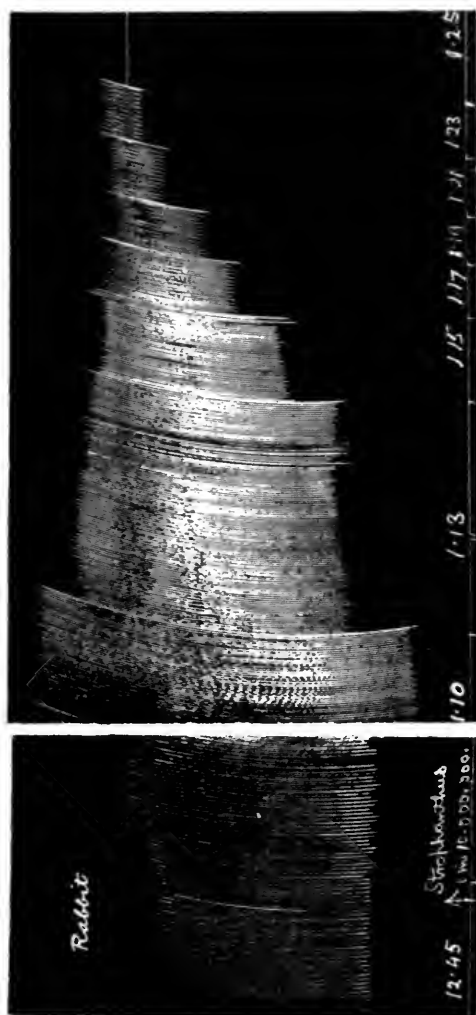


FIG. 1. ISOLATED HEART OF RABBIT

Showing effect of strophanthus extract, 1 in 10,000,000. The heart is arrested in systole in forty-five minutes. In this and the following figure the record was stopped at intervals to save space.

clusion that the congenital tolerance of the rat to strophanthus is due chiefly, if not entirely, to an insusceptibility of the heart muscle of this animal to the action of strophanthus.

The fact that the differences in resistance of the isolated hearts of the rat and the rabbit are less marked when strong solutions are used may possibly be correlated with the fact that the minimum lethal dose in the rat, once it is reached, kills almost as quickly as the (smaller) minimum lethal dose kills the rabbit. Thus though the ratio in the case of concentrations of strophanthus which arrest the heart slowly is: rabbit : rat = 1 : 20 to 40, the ratio of stronger solutions is only: rabbit : rat = 1 : 2, because a 1 in 250,000 solution arrests the rat's heart in the same time as a 1 in 500,000 solution arrests the rabbit's heart.

#### C. ACTION ON RED BLOOD CORPUSCLES

It being found that the rat's heart shows a specific insusceptibility to the action of strophanthus, a further question arises as to whether tissues of the rat other than the heart share this property. It was hoped that some light might be thrown upon this by the action of strophanthus on the red blood corpuscles. Most glucosides of the digitalis group have the property, to a greater or less extent, of haemolysing red blood corpuscles. It was interesting, therefore, to determine whether the rat's corpuscles show a greater resistance than the rabbit's corpuscles to the haemolytic action of strophanthus. Unfortunately, no definite decision could be obtained from this method of enquiry, so that it is unnecessary to give the experiments in detail. It was found, however, that there is no difference in the resistance to strophanthus-haemolysis between rabbit's and rat's corpuscles. If a one per cent suspension of washed corpuscles were used, it required a minimum strength of solution of 1 in 2000 of strophanthus extract to haemolyse the corpuscles of both animals within four hours. Any conclusion, relative to this enquiry, which can be drawn from those haemolysis experiments, is rendered unreliable for two reasons: (1) the concentrations required to arrest the heart and to haemolyse corpuscles are so widely different that



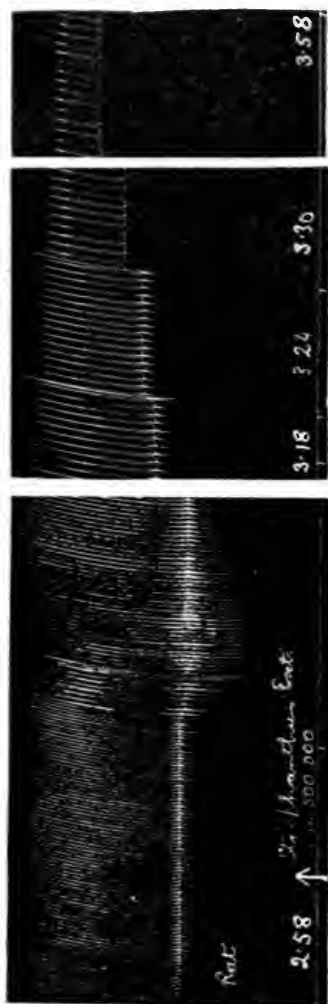


FIG. 2. ISOLATED HEART OF RAT

Showing effect of strophanthus extract, 1 in 500,000. The heart is not arrested in sixty minutes. The effect is less than that produced by a solution twenty times weaker on the rabbit's heart (cf. fig. 1).

one cannot be sure that the actions on the heart and on the red blood corpuscles have anything in common; (2) even the slight haemolytic effect produced by strophanthus extract may not be due to the same substance as acts on the heart, because crystalline strophanthin (Thoms) was found to have no haemolytic action on either rabbit's or rat's corpuscles.

#### DISCUSSION

It was pointed out long ago by Vulpian<sup>2</sup> that the toad is insusceptible to its own poison. Later<sup>3</sup> he advanced the hypothesis that this insusceptibility is due to "a sort of accustoming produced by continued molecular absorption of the toxic humor." He considered this more probable than that "the muscular tissue (heart) contains the reason of its immunity to the toad's own poison." In the same communication he pointed out that the toad heart, unlike that of the frog, is not affected by digitalis, which has an action on the heart similar to toad poison.

Since Vulpian's time further facts have been added. Thus both Faust<sup>4</sup> and Abel and Macht<sup>5</sup> have shown that the toad's skin contains an active principle chemically and pharmacologically allied to the active principles of digitalis. It has been found, too, that the toad heart is less susceptible than the frog's heart not only to toad poison and to digitalin, but also to strophanthin and antiarin and apocynamarin. This insusceptibility of the toad heart to glucosides of the digitalis group has always been associated in a vague way with the fact that the toad secretes, and probably absorbs, a poison which has a similar physiological action.

Now the rat, so far as is known, secretes no cardiac poison; but it also shows a partial tolerance to glucosides of the digitalis group. It is possible, therefore, (1) that such a tolerance may exist without the natural absorption of a cardiac poison, and (2)

<sup>2</sup> Vulpian: C. R. Soc. de Biol., iii, p. 125, 1856.

<sup>3</sup> Vulpian: C. R. Soc. de Biol., v, p. 115, 1858.

<sup>4</sup> Faust; Archiv of Exper. Path. u. Pharm., xlvii, p. 278, 1902.

<sup>5</sup> Abel and Macht: This Journal iii, p. 319, 1912.

that, in contrast to Vulpian's conclusion, the muscular tissue of the heart (as shown by these experiments) may contain the reason of its own tolerance. If this is possible in the rat, it may also be possible in the toad. This investigation, therefore, suggests the possibility that the tolerance of the toad to digitalis glucosides may be an accidental concomitant of the fact that the toad secretes a digitalis-like acting poison.

#### SUMMARY

1. The minimum lethal dose by subcutaneous injection of an extract of strophanthus was found to be thirty times greater for the rat than for the rabbit.
2. A solution of this extract, twenty to forty times stronger, is required to arrest the isolated rat's heart than suffices to arrest the isolated rabbit's heart in the same time.
3. The congenital tolerance of the rat to strophanthus can therefore be explained by the fact that the rat's heart itself is insusceptible to the action of strophanthus.
4. The tolerance of the toad's heart to digitalis-like poisons may be unconnected with the fact that the toad secretes a poison which has a similar cardiac action.



## MODE OF UNION BETWEEN THE AMANITA-HAEMOLYSIN AND ITS ANTIHAEMOLYSIN

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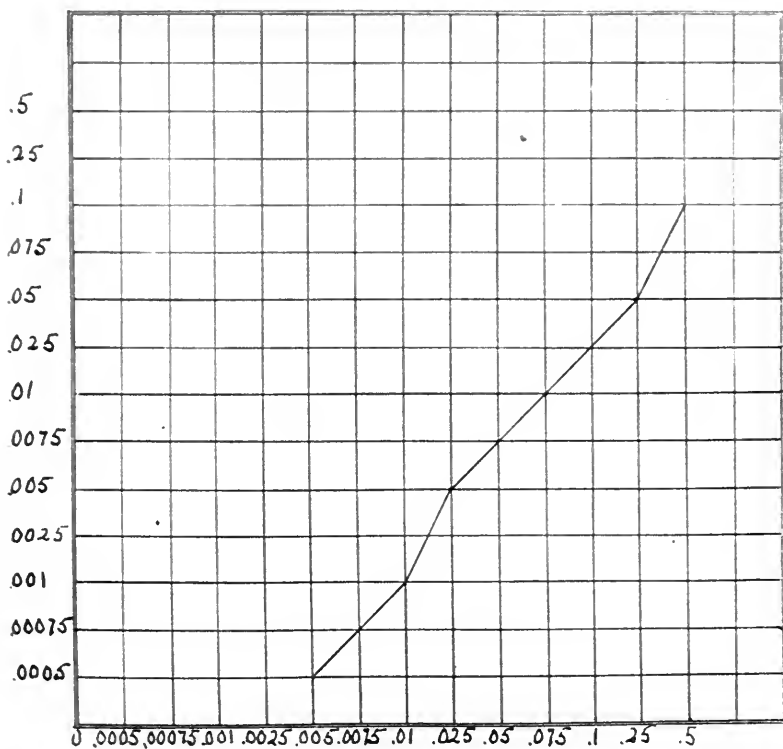
On various occasions (1) it has been pointed out that the subcutaneous introduction of graduated doses of aqueous extracts of *Amanita phalloides* into animals causes the production of an antiserum with antitoxic and antihaemolytic properties. An antihaemolysin with a strength of 1-1000 or 1-2000 can easily be obtained using as an index that quantity of a haemolytic extract which will completely dissolve one cubic centimeter of a 5 per cent suspension of rabbit's blood. Despite the marked antihaemolytic properties of the sera their antitoxic power is always extremely low. This contradiction has now been explained by experiments with the *Amanita-toxin* and the *Amanita-haemolysin* completely separated from each other (2). These experiments indicate that while animals react quickly and easily towards the haemolysin producing a characteristic antiserum, they can be immunised to the toxin only with the greatest difficulty and at best are able to withstand the introduction of but small multiples of a fatal dose. The observations made by Abel and Ford (3) in regard to the chemical nature of the *Amanita-haemolysin*, which from their investigations appears to be a very sensitive glucoside<sup>1</sup> attach great importance to the properties of the anti-

<sup>1</sup> Rabe has recently published a paper from Kobert's laboratory in which he maintains the original position taken by Kobert that the haemolytic substance in *Amanita phalloides* (*Phallin* or *Amanita-haemolysin*) is a toxalbumin. An answer to Rabe's contention is in course of preparation and will be published shortly. It should be mentioned here, however, that Rabe has brought forward no new facts and in our opinion has presented no satisfactory evidence to disprove the conclusions of Abel and Ford, that all material of a proteid nature may be removed from the *Amanita-haemolysin* without materially reducing its haemolytic activity and that this proteid-free haemolysin gives the reactions of a glucoside. See Rabe, *Zeitschr. f. exper. Path. u. Pharm.*, Bd. 9, H. 2, S. 352.

haemolysin and we have therefore been particularly interested in its study. The antihaemolytic sera which we have examined have been usually obtained from rabbits treated with whole extracts of the fungus or with solutions of the haemolysin freed from toxin. One of the best and most powerful sera, however, was obtained from a horse treated with the fungus extract by Dr. Kinyoun in the Mulford Laboratories. This serum had originally, in January, 1907, an antihaemolytic strength of 1-2000. It has since been kept on ice and at various times used for experiments. When last tested in 1910 the serum, then about three years old, had an antihaemolytic strength of 1-500. At this time we made the attempt to gain information as to the mode of union between the *Amanita-haemolysin* and its antihaemolysin using this serum in the investigation, and a series of experiments was outlined and begun. This work was unavoidably interrupted before it could be brought to a satisfactory conclusion. Certain interesting reactions were obtained in the early observations, however, which throw some light upon the problem and it seems worth while to call attention to them at the present time.

If a fresh extract of *Amanita phalloides* be made, filtered through a Berkfeld filter, rendered isotonic by the addition of NaCl, the haemolytic strength of this solution can be determined with great exactness and correspondingly the exact quantity of the antihaemolytic serum which will neutralise it, that is, produce a mixture which will have no solvent action on blood corpuscles. If now increasing quantities of the haemolysin be employed and the amount of antihaemolysin necessary for neutralisation be determined the increase in the antihaemolysin can be found. By plotting the data obtained by means of a system of abscissas and ordinates the manner in which the haemolysin and antihaemolysin combine can be represented in a graphic way and exact information obtained as to the law which holds for the union of the two. A similar series of observations starting with fixed quantities of antihaemolysin and estimating the amount of haemolysin neutralised can also be made and in turn plotted. We thus obtain two lines or curves showing the combining power of the haemolysin and antihaemolysin. Such data

have been obtained and plotted, the results being shown in Charts I and II. By reference to them it may be seen that the mode of union is apparently of the simplest character, following the law of multiple proportions. If we start with the haemolysin



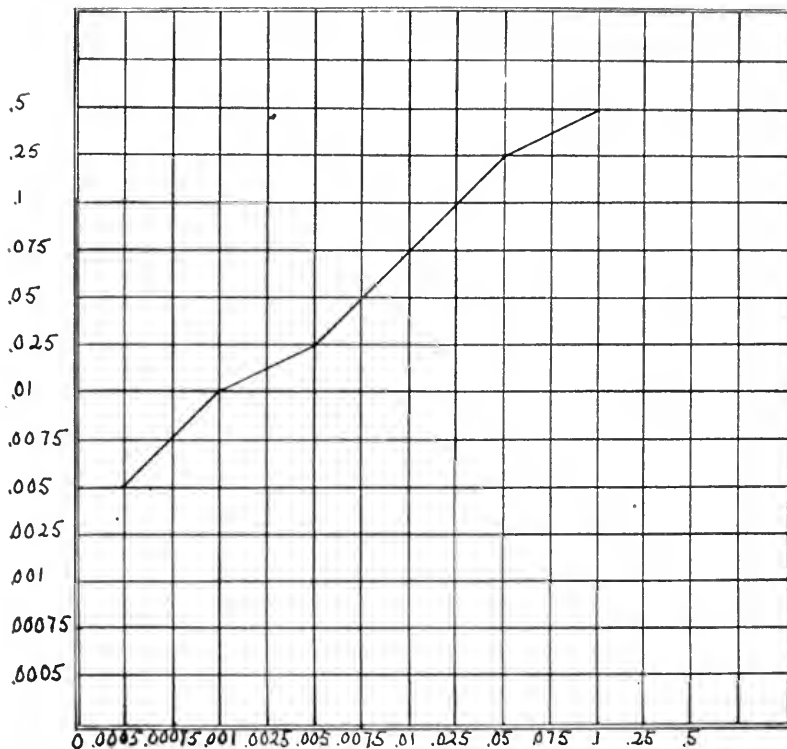
Abcissa = number of cubic centimeters of haemolysin employed

Ordinate = number of cubic centimeters of antihaemolysin employed

CHART I. NEUTRALISATION OF HAEMOLYSIN BY ANTIHAEMOLYSIN

and increase its quantity by simple multiples, the quantity of antihaemolysin increases also by similar multiples (Chart I). In the same way starting with the antihaemolysin the haemolysin is found to increase proportionally (Chart II). In both cases the mode of union is in the form of a straight line and the law

governing it, that of simple multiple proportions. It should be noted that such curves are to be obtained only with *fresh* extracts of *Amanita phalloides* and in no wise affect the question as to whether partially degenerated haemolysins or extracts robbed of some of their haemolytic activity by the action of heat or acids



Abscissa = number of cubic centimeters of antihaemolysin employed

Ordinate = number of cubic centimeters of haemolysin employed

CHART II. NEUTRALISATION OF ANTIHAEMOLYSIN BY HAEMOLYSIN

have the same combining power in their neutralisation by the antihaemolysin. Indeed certain observations which we have made seem to indicate that different curves may be obtained from such extracts, but for fresh haemolysins the mode of union is clearly such as is represented on the charts.



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## NOTE ON THE AMANITA-TOXIN

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It has been shown (1) by a number of previous investigations that the poisonous fungus *Amanita phalloides* contains two toxic substances to one of which we have given the name *Amanita-haemolysin* and to the other the name *Amanita-toxin*. The haemolysin was originally described by Kobert (2) under the name Phallin and was at first regarded by him as the active principle of the plant in cases of human intoxication. It has subsequently been found (3) that this haemolytic body is a glucoside and not a toxalbumin as first supposed by Kobert. Because of the rapidity with which it is destroyed by heat (65°C. one half hour) and by the action of pepsin and pancreatin but little importance can be assigned to it in cases of poisoning by this fungus. We regard the *Amanita-toxin* as the active principle chiefly because of its resistance to the action of heat and the digestive ferments and because of its ability to produce lesions in animals essentially identical with those described for man. The profound fatty degeneration produced by the fungus attaches great interest to any chemical investigations into the nature of this poisonous substance which from its extreme toxicity ranks as one of the most powerful poisons of plant origin known. At various times methods for its isolation and purification have been devised (4). It is highly soluble in 65 per cent alcohol, can be precipitated by phosphotungstic acid (10 per cent phosphotungstic acid in 5 per cent sulphuric) and freed from impurities by the use of silver nitrate (15 per cent solution) and basic lead acetate (saturated solution) both of these reagents throwing down voluminous precipitates non-toxic in character. Its failure to reduce Fehling's solution even after prolonged boiling in 5 or 10 per cent hydro-

chloric acid indicates that it is not a glucoside, at least in the ordinary sense of the term and at no time have we been able to obtain from it alcaloidal color reactions or reactions for proteid or conjugate sulphate. We have therefore been unable to characterise this substance definitely and are quite unwilling to subscribe to the view recently expressed by Rabe (5) that it is alcaloidal in character. Indeed it is difficult to see upon what evidence Rabe bases his conclusions since the reactions which he found were not apparently obtained from a purified toxin. As Schlesinger and Ford (loc. cit.) have pointed out, the toxin purified by their method gave no tests for alcaloids.

Recently in the hope of obtaining more information as to the nature of this *Amanita-toxin* we have prepared a small quantity and submitted it to combustion analysis. For this purpose we extracted 200 grams of the thoroughly dried fungus with alcohol and isolated the poison from this solution by the method of Schlesinger and Ford (loc. cit.) At every step in the procedure the various products were tested to determine their activity. The final solution had a high degree of toxicity 1 cc. containing 0.0007 grams of material killing a guinea pig weighing 175 grams in seven hours. This solution was then evaporated to dryness on a water bath, taken up in water and precipitated in absolute alcohol in which reagent the purified toxin is practically insoluble. This last precipitate was dried in a vacuum dessicator. From the 200 grams of fungus we obtained 0.2114 grams of a slightly pigmented amorphous powder which represents the active principle and retained its toxicity unimpaired. This substance has failed to crystallise under any conditions which we have thus far been able to produce. Its amorphous character renders investigations into its chemical nature extremely difficult and the results of combustion analysis are necessarily of doubtful value. The final product, however, had such a high degree of toxicity and was the result of such rigorous methods of purification that we considered it worth while to make such an analysis which gave us the following results calculated for ash-free substance. The ash content was but 9.5 per cent and consisted mainly of sodium salts.

	COMBUSTION ANALYSIS OF <i>Amanita-toxin</i>		
	I	II	III
C.....	42.89	43.46	
H.....	6.79	7.29	
N.....			14.16
S.....		2.91	

In view of the work of Abel and Ford (loc. cit.) on the *Amanita-haemolysin* which they found to contain nitrogen and sulphur, it is interesting to note that here also our final product contains these substances in considerable quantity. The haemolysin and the toxin differ radically not only in their physiological properties, but in their chemical reactions and require different methods of preparation. The haemolysin, for instance, is precipitated by 65 per cent alcohol and by basic lead acetate while the toxin is soluble in alcohol of this strength and is not thrown down by lead acetate. Many other chemical differences are to be noted. In spite, however, of the dissimilar methods of preparation the two final products, in both cases physiologically active, contain nitrogen and sulphur, in addition to the elements carbon, hydrogen and oxygen.

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## NOTE ON THE ACTION OF HISTAMIN UPON SURVIVING ARTERIES

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Histamin,<sup>1</sup> an active principle of ergot, is a base produced by splitting off carbon dioxide from histidin by chemical or bacterial agents. Dale and Laidlaw (1) who have shown that it is in general a stimulant of plain muscle state that the uterus and bronchioles are especially sensitive to this substance, the systemic arterioles less so. They have found in some species, chiefly carnivora, an unexplained antagonistic peripheral action which overcomes the vaso-constrictor effect of histamin; the blood pressure instead of being raised shows a marked fall. This fall in association with lowered temperature, bronchial spasm and general signs of collapse presents a picture which has been compared to that of "anaphylactic shock." The vaso-dilation is ascribed by Dale and Laidlaw to "a nervous mechanism producing an antagonism which does not survive the conditions of perfusion," for isolated perfused organs of cat and dog give only a diminished flow under histamin. Degeneration experiments have shown that the sympathetic neurones are not essential to the antagonistic vaso-dilation, and a similar level of action to that of epinephrin is therefore suggested for this substance.

Dale and Laidlaw show further that although it may act at a similar level histamin does not inhibit the plain muscle (bronchioles and uterus of non-pregnant cat) which epinephrin inhibits.

A number of experiments reported briefly here were made upon isolated rings of surviving mammalian arteries. They confirm

<sup>1</sup> Beta-iminazolyethylamin. The hydrochlorides of histamin and of tyramin used in these experiments were the products of a well-known German firm.

the interesting observation of Dale and Laidlaw that isolation of a vessel seems to preclude the possibility of a vaso-dilator response to histamin, and show further that there is another example of plain muscle inhibited by epinephrin which histamin does not inhibit, but contracts, namely, the coronary artery of the ox.

The fresh artery rings were suspended in 10 cc. of Ringer's solution and a solution of 0.1 per cent histamin hydrochloride was added after removal of the initial tonus; 0.25 to 2 mgs. of histamin were given at one dose. The hydrochloride has a faintly acid reaction but in a number of experiments the solution was carefully neutralized with weak sodium hydroxide and gave confirmatory results. The contraction of the vessel ring was in every case prompt and striking (except in the case of the cerebral arteries which also respond to epinephrin in a questionable manner). The following results were obtained:

SPECIES	ARTERY	NUMBER OF CASES	EFFECT OF HISTAMIN
Ox.....	carotid	4	contraction
	mesenteric	2	contraction
	coronary	4	contraction
Calf.....	cerebral	2	(?)
Dog.....	cerebral	3	{ 1 contraction 2 others (?)
	carotid	1	contraction
Cat.....	mesenteric	1	contraction
	carotid	6	contraction
Rabbit*.....	mesenteric	1	contraction
	pulmonary	5	contraction

\*No antagonistic vasodilation has been described for the intact animal of this species.

After some minutes 1 or 2 mg. of pilocarpin were added and the normal result was obtained, i.e., contraction of the coronaries or dilation of the other vessels. This was followed after another short interval by 0.1 mg. of epinephrin which gave dilation of the coronaries or constriction of the other vessels, as usually described for these animals (see fig. 1).

The action of histamin on the ox coronary is of especial interest because of its variance with epinephrin in this case. The



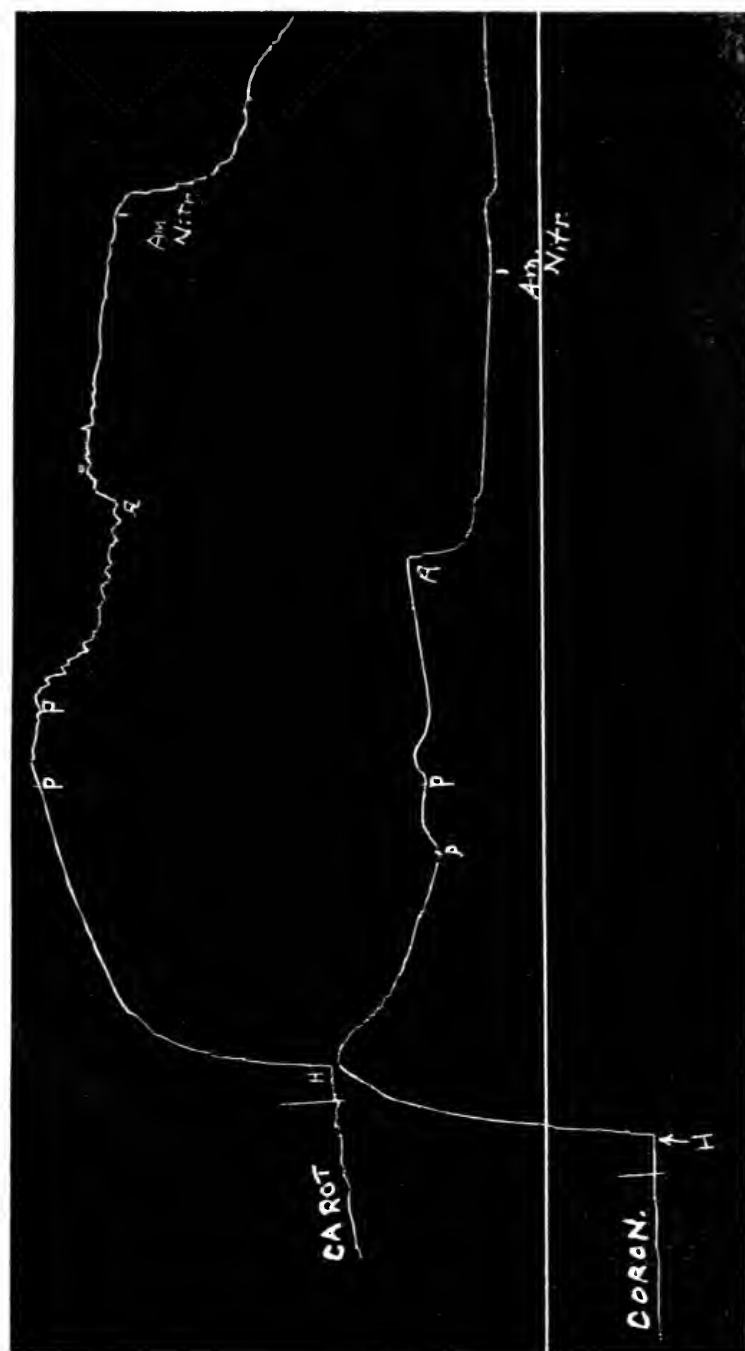


FIG. 1. RINGS OF CAROTID (UPPER TRACING) AND CORONARY (LOWER TRACING) ARTERIES OF OX SUSPENDED IN SAME RINGER BATH. At 5.53 p.m. 0.5 cc. of 0.2 per cent histamin (H) (exactly neutralized) was added; at 6.13 and 6.18 p.m. 1 and 2 cc. respectively of 0.2 per cent pilocarpin (P & P); at 6.32 p.m. 0.1 cc. of 0.1 per cent epinephrin (A); at 6.54 p.m. 0.5 cc. of amyl nitrite. Up-stroke = contraction.

epinephrin dilation is generally regarded as due to the innervation of this artery with dilators rather than constrictors of sympathetic origin (a condition apparently not existing in the human coronary) (2). Park and Janeway (3) have made of the ox coronary a good test object for distinguishing between epinephrin and other vaso-constrictor substances, e.g. those of blood serum. The accompanying figures show that histamin is properly classed (O'Connor) (4) with those substances which increase the tone of all smooth muscle rather than with those (epinephrin) which have a selective action upon the myoneural junction of true sympathetic nerves (Langley). This nervous mechanism is proven to survive the conditions of our experiments and appears to possess no affinity for histamin.

This vaso-constriction of the coronary rings by histamin is corroborated by the perfusion experiments of several authors. Dale and Laidlaw in the rabbit's heart obtained a decrease in coronary outflow. In the living dog F. Meyer (5) finds after giving histamin intravenously a decreased flow from the coronary veins which he attributes to the fall in general blood pressure, (not having investigated the factor of local vaso-constriction). Rabe (6) working on three cats' hearts obtained in two a decrease in coronary flow after histamin but in the other a 50 per cent increase.

Tyramin, p. hydroxyphenylethylamin, another basic principle of ergot (obtained by splitting off carbon dioxide from tyrosin) has been termed "sympathomimetic" by Dale and Dixon (7) who found that in addition to striking vaso-constrictor properties this substance also resembles epinephrin in inhibiting the tonus of the non-pregnant cat uterus. But the analogy between tyramin and epinephrin fails when we apply this substance to the ox coronary, for vaso-constriction only has been obtained in all three cases tested. One of these cases is shown in figure 2. Tyramin may therefore be regarded as occupying a pharmacological position midway between epinephrin and histamin, and perhaps we may assume also that a chemical (or physical chemical) difference is to be looked for between the receptive substance at the myoneural junction of the inhibitory nerves of the non-

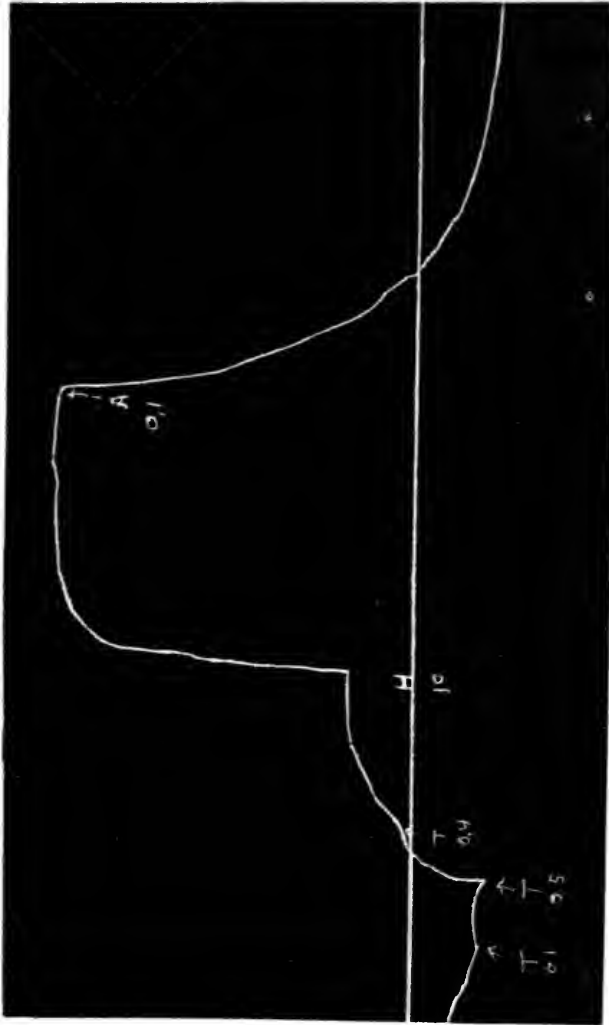


FIG. 2. RING OF OX CORONARY

At 4.46, 4.49 and 4.54 p.m. were added respectively 0.1, 0.5 and 0.4 cc. of 0.1 per cent tyramin (T, T and T); at 5.03 p.m. 0.5 cc. of 0.2 per cent histamin (H); at 5.20 p.m. 0.1 cc. of 0.1 per cent epinephrin (A). Up-stroke = contraction.

pregnant cat uterus and the corresponding substance of the inhibitory mechanism of the ox coronary, although both are of true sympathetic origin.

The following scheme indicates some of the relations between epinephrin, tyramin and histamin, + standing for blood pressure rise or muscle contraction and (—) standing for fall of blood pressure or muscle dilation.

ACTION ON	BLOOD PRESSURE	PERIPHERAL VESSELS	OX CORONARY	NON-PREGNANT CAT UTERUS
Epinephrin.....	+	+	(—)	(—)
Tyramin.....	+	+	+	(—)
Histamin.....	(—)*	+	+	+

\* Some species, chiefly carnivora (Dale and Laidlaw).

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## THE PHARMACOLOGICAL ACTION OF CATHA EDULIS AND ITS ALKALOIDS

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The leaves of the *Catha edulis*, under the name of Kat, have been extensively used for centuries as a stimulant-narcotic in Abyssinia, Arabia, and Somaliland, and since the time of the Swedish botanist and traveller Forskal, who first described them in his *Flora Ægyptiaco-Arabica* (published in 1775) they have been frequently noticed by other European writers. The fresh and dried leaf of the plant is chewed like coca, or is made into an infusion and drunk like tea, when it produces a feeling of refreshment, and either a stimulant or a sedative effect, much in the same manner as is so widely recognised with the better-known coca, tobacco, opium, and other substances of a like nature. Several previous attempts have been made to isolate the active principles, hitherto without much success, but I have succeeded in obtaining pure from the leaves and wood three alkaloids which I have named Cathin, Cathinin, and Cathidin, of which I have given an account elsewhere.<sup>1</sup> For a detailed description of the methods of extraction, solubilities, and reactions of these, I must refer to my other paper, as I propose to give here chiefly an account of the more striking and obvious pharmacological actions of the three alkaloids and of infusions made from the leaf and wood. It is probable that other alkaloids, or at least decomposition products of others, can be got both from the leaves and wood.

I obtained the alkaloids from the powdered leaf or wood by macerating in acidulated water, making the expressed liquid alkaline with

<sup>1</sup> *Pharmaceutical Journal*, p. 676. November 30, 1912.

sodium carbonate, and extracting with chloroform. On distilling off the chloroform and taking up the residue with very dilute sulphuric acid impure cathin sulphate is obtained. This is dried over sulphuric acid and then washed with absolute alcohol in which it is insoluble.

The leaves are then made alkaline with sodium carbonate solution and macerated in ether of 0.730 specific gravity. On distilling off the ether a bulky green resinous mass is left, and from this the cathidin can be extracted with 5 per cent hydrochloric acid, from which it is precipitated by ammonia or sodium carbonate solution. It is easily purified. Cathinin is obtained from the alkaline filtrate and washings by shaking with chloroform, distilling off the chloroform, neutralising with dilute sulphuric acid, drying thoroughly over sulphuric acid, and washing the sulphate with chloroform in which it is insoluble.

Cathin sulphate, when crystallised from its watery solution, is in small needle-shaped crystals, its reaction is neutral, and it has a bitter taste. It gives precipitates with phosphomolybdic acid, Bouehardat's reagent (I in KI), Mayer's reagent ( $\text{HgCl}_2$  in KI), and picric acid, but not with platinic chloride, tannic acid, auric chloride, mercuric chloride, potassium hydrate, ammonium hydrate or sodium carbonate solution. It is easily soluble in water and in dilute alcohol, but it is insoluble in absolute alcohol, chloroform, ether, benzol, acetic ether, petroleum ether, acetone, and amyllic alcohol, and very slightly soluble in 90 per cent alcohol, more so in 50 per cent.

If cathin sulphate be dissolved in water and the solution made alkaline with sodium carbonate and shaken out with chloroform, the chloroform leaves on evaporation small needle-shaped and feathery crystals mixed with more or less gummy alkaloid. The base thus obtained is very soluble in water, and has a bitter taste. It is insoluble in ether and petroleum ether, but very soluble in chloroform, absolute and diluted alcohol, benzol, amyllic alcohol, and acetone. The treatment with alkali and chloroform seems to cause a certain amount of decomposition of the alkaloid, as its watery solution (in contradistinction to that of the sulphate) gave precipitates with tannic acid, auric chloride, and platinic chloride, soluble in excess of the reagents. Otherwise its behaviour to reagents is the same as that of the sulphate. Its physiological action was also altered somewhat as compared with the sulphate.

Cathidin is a white amorphous powder, insoluble in cold and hot water and in petroleum ether, but freely soluble in ether, absolute alcohol, acetone, chloroform, acetic ether, benzol, toluol, and xylol, somewhat less so in amyllic alcohol. The addition of water precipitates

it from its solutions in acetone and in alcohol, and it is soluble, but not freely, in 40 per cent alcohol. It does not dissolve readily in 1 per cent solution of hydrochloric or nitric acid, nor in weak tartaric acid, but the addition of a little more strong acid dissolves it freely. It dissolves in hydrochloric, nitric, and sulphuric acids without any change of colour, but is very slightly soluble in strong acetic acid. When dissolved in an acid and dried *in vacuo* it does not form crystalline salts, and the addition of water precipitates the base. It is, therefore, a very weak base. Its solution in acid has a very bitter taste. From acid solution it is precipitated by caustic alkalies and their carbonates and bicarbonates, and by calcium hydrate and ammonium hydrate. The precipitate is soluble in excess of potassium and ammonium hydrates and in large excess of sodium carbonate. Left in contact with these alkalies, it seems gradually to decompose. Its acid solution gives dense precipitates with platinic chloride, mercuric chloride, auric chloride, picric acid, phosphomolybdic acid, and the other common reagents for alkaloids.

Cathinin sulphate crystallises from water in rosettes of needle-shaped or feathery crystals. It is easily soluble in cold water (with a bitter taste) and with difficulty in a large amount of absolute alcohol, it is insoluble in ether, chloroform, acetone, etc. Its watery solution gives dense precipitates with phosphomolybdic acid, Bouchardat's reagent, Mayer's reagent, platinic chloride, tannic acid, picric acid, auric chloride, and white precipitates readily soluble in excess with potassium hydrate, ammonium hydrate, and sodium carbonate solution. Cathinin can be obtained from the solution of its sulphate by adding an alkali and extracting with chloroform. It leaves a colourless semi-crystalline or amorphous gummy residue not very soluble in cold water, the solution having a very strong alkaline reaction and a bitter taste. It is much more soluble in hot water than in cold, and is very soluble in acetone, absolute and dilute alcohol, acetic ether, chloroform, and amylic alcohol, soluble in a fairly large amount of ether, scarcely soluble in benzol and not in petroleum ether.

There are only two previous researches which deal with the action on lower animals of alkaloids obtained from catha leaves. U. Mosso<sup>2</sup> isolated a small quantity of an impure alkaloidal substance soluble in ether, which he named celsastrin. It acted as an excitant to the nervous system in small doses, and as a depressant in large doses. He makes

<sup>2</sup> Rivista clinica, xxx, 65, 1891.

no mention of any action on voluntary muscle. It could not have been cathin or cathinin, as it was much more powerful than either, and 0.05 gram killed a rabbit weighing 1730 grams. Besides, it was extracted by a method which would inevitably decompose either of these alkaloids and compared with them it has different solubilities and reactions. It was not nearly so active as the fourth alkaloid which I obtained (from D. B. Dott) but have not named. Following Mosso's process exactly I was unable to isolate it from the leaves.

The other research is by Chevalier<sup>3</sup> and I regret that my attention was not drawn to it until my paper on the chemistry of *Catha edulis* appeared in the *Pharmaceutical Journal*. He states that Beittner in 1900 published a description of an alkaloid obtained from the leaves which was named *Katine*. He himself obtained a quantity of it, slightly modifying Beittner's method of extraction. It is precipitated from its acid solution by ammonia, and is very slightly soluble in water, ether, and petroleum ether, but easily in alcohol and chloroform. Ammonia and heat were freely used in its extraction, and this is sufficient to decompose cathin and cathinin. Chevalier states that this alkaloid acts chiefly on the nervous system, and adds "En particulier l'action désorganisant de la caféine sur le muscle, déterminant rapidement la contraction et l'inexcitabilité manque totalement." But all the alkaloids which I obtained had a most marked effect on voluntary muscle causing rigidity and rigor mortis. Further the fresh infusion made from the leaves or wood has this action in a marked degree. I am unable to identify this alkaloid with any of those which I have described. It is absolutely essential not to use heat or strong acids or alkalis in the processes of extraction, otherwise decomposition ensues.

After reading Chevalier's paper I re-worked a small quantity of leaves which I had by me. The powdered leaf was mixed with calcium hydrate and water, then extracted with hot water, and the liquid expressed, filtered and shaken up with chloroform. On distilling off the bulk of the chloroform and evaporating the last part, neutralising the residue with dilute sulphuric acid, drying, and washing with absolute alcohol, pure crystals are left which are identical with the alkaloid which I have described as cathidin sulphate. At present I am investigating the chemistry of cathidin and certain other matters, and hope soon to be in a position to publish a further communication on the whole subject.

<sup>3</sup> Bull. gén. de Thérapeutique, 161, 572, 1911.



## ACTION OF THE LEAVES

For the purpose of obtaining a general idea of the action of the crude drug I first studied on frogs and cats the effects of an infusion made by pouring boiling water over the finely powdered leaf and then expressing the liquid. It was always made of such a strength that 1 cc. of the infusion corresponded to 0.5 gram leaf or wood. Both leaf and wood had the same action, and the dose required of each was the same.

*Frogs.* When 1 cc. was given subcutaneously, the frog in a few minutes became slightly somnolent and somewhat clumsier in its movements, and its pupils contracted a little. The brain and spinal cord were evidently somewhat depressed. During the next hour the animal gradually became paralysed, and ultimately ceased to react to pinching. The pupil might either dilate or remain rather small, and any eye-reflex was ultimately abolished. At this stage the sciatic nerves gave no reaction to strong faradic stimuli, the muscles at the place of injection also did not react, while the other voluntary muscles reacted quite normally. Later the heart stopped in diastole. Doses up to 2 cc. (equal to 1 gram leaf) had the same action, death taking place in two or three hours with the heart stopped in diastole, most of the muscles being in rigor mortis and the peripheral nerves paralyzed. The pupil was small at first but dilated later on. With descending doses (equal to 0.25 gram to 0.1 gram leaf) the pupil usually dilated from the beginning, somnolence was very slight, and after some hours there occurred a slight but quite distinct increase of the spinal reflexes, which continued for the next day or two. At this stage the animal could jump actively and vigorously. On the third or fourth day the movements became gradually feebler, and death took place apparently from a poisoning of the voluntary muscles, which was most marked at the place where the injection was made, but which extended more or less to the other muscles.

When medium doses were given *per os* the muscles were not visibly affected, the only result being a very slight increase of the

reflexes, and the frog remained very active and lively in its movements.

*Cats.* The action of a watery infusion was very slight. Thirty grams of the powdered leaf were macerated in 70 cc. boiling water, the liquid expressed, and given to a small cat by a stomach tube. The pupils dilated and there was some diarrhoea, but no marked symptoms ensued. An infusion made from a similar amount of the wood and evaporated down to the consistence of a syrup, had absolutely no visible action on a rabbit when given by the stomach, but probably the active principles were decomposed by the heating.

#### ACTION OF CATHIN

Cathin sulphate in pure crystals was given hypodermically dissolved in water to frogs and cats.

*Frogs.* The symptoms produced in frogs depend very largely on the dose, but the action is essentially the same no matter what dose is given, and depends on poisoning of the central nervous system and voluntary muscles. One centigram (0.01 gram) may be regarded as a medium dose for a large frog (*R. temporaria*). When this amount is given the pupils usually dilate almost at once, and in ten to twenty minutes the movements have become clumsier and heavier, and the animal is slightly somnolent. It crawls about rather than jumps, and at this time there may be a slight increase of reflexes, while the muscles in the neighbourhood of the injection are stiff. Next day the animal is alert and active, but stiff and clumsy in its movements and the slight increase of reflexes is more apparent. The pupil is fully dilated. It remains in this condition for two or three days and then recovers. With small frogs this amount is fatal.

Two centigrams (0.02 gram) is a large dose. When it is given the pupils may contract or dilate almost at once, and in five or ten minutes the animal becomes duller, ceases to jump, and soon after lies flaccid, responds to pinching only by a twitch, and does not wink if the eyeball be touched. Respiration ceases early, the skin reflexes are lost, and gradually the peripheral nerves become paralyzed and the muscles at the place of injection

tion respond not at all or feebly to electric stimulation. The frog may gradually recover, and as the peripheral nerves regain their function a more or less marked increase of spinal reflexes becomes developed. Recovery takes several days. After this dose the frog usually dies, when the heart is found stopped in diastole, most of the muscles are completely irresponsive to electric stimulation, and the motor nerves are paralyzed.

With small doses (2 to 5 mgms.) the pupils dilate, the somnolent stage is ill-marked, and in two or three hours the reflexes are slightly increased. For four or five days the frog is very lively and active with slightly increased reflexes, and then it returns to normal. The action was the same with *Rana esculenta*.

In a decerebrate frog the aorta was tied low down to protect the sciatic nerves, and 2 mgms. cathin sulphate dissolved in water was injected by a cannula passed through the ventricle and tied into the aorta. The spinal cord was paralysed almost at once without any stage of increased reflexes. The muscles of the legs and the sciatic nerves remained quite excitable to electric stimuli.

*Cats.* A dose of 0.05 gram cathin sulphate given hypodermically to a young cat (870 grams) caused dilatation of the pupils, vomiting, and considerable restlessness and excitement for some hours.

Another cat (1060 grams) after 0.1 gram had almost immediately fully dilated pupils, became very quiet for about fifteen minutes and then began to be restless and to salivate profusely. In an hour it was turning its head restlessly, starting, staring about, and had tremors almost amounting to slight spasms. There was no increase of reflexes on stroking its skin, but when it jumped it gave a violent jerk on landing. This continued for five or six hours, but by next day it had recovered with the exception of widely dilated pupils. When it was most affected there was distinct paresis of the hind legs.

Another medium-sized cat received 0.2 gram hypodermically. Almost at once the pupils dilated, it began to salivate profusely and in a few minutes it was nervously glancing about and starting violently on the least noise. In about three-quarters of an

hour the symptoms of cerebral excitement were very evident, as shown by twitching of the ears and tail, tenseness of the claws, and extreme restlessness. Half-an-hour later there were intervals of rapid and panting respiration, the hind legs were paretic, and the head tremulous. Shortly after it could not stand, but kept stumbling about continuously and restlessly. The pupils were fully dilated. The paresis rapidly increased, there was great dyspnoea, and although it could not stand, it rolled about and tried to move constantly. The sensory functions were maintained apparently unimpaired, and if there was any increase of the spinal reflexes it was completely concealed by the paretic condition of the peripheral motor nerves. The cat remained in this state all day, but recovered completely during the night, although the pupils still kept fully dilated. During the whole time the rate and force of the heart were scarcely affected.

It is evident that the actions of cathin sulphate and of an infusion of the leaves or wood are almost exactly similar. In frogs when large doses are given subcutaneously, they act as intense muscle poisons, and at the same time paralyse the brain, spinal cord, and peripheral motor nerves: with smaller doses the depressant action on the nervous system is not nearly so marked and is succeeded by a slight increase of reflex excitability. Proportionately the leaves are much more powerful than the alkaloid, but this may be accounted for by other alkaloids being present. In cats the action of cathin is chiefly that of a cerebral excitant, but with large doses the peripheral motor nerves are depressed, the spinal cord has its reflex excitability increased, and the muscles are also affected but not to such a serious extent as in the frog.

#### ACTION OF CATHININ

Cathinin sulphate in white crystals, readily soluble in water with a neutral reaction, was given hypodermically.

*Frogs.* When administered to frogs in a dose of 0.01 gram no narcosis or somnolence occurred, the pupils rapidly dilated, and in fifteen minutes or thereabouts the spinal reflexes had become greatly increased, tetanic spasms being elicited on stimulation.

In an hour or so the motor nerves gradually became paralyzed and the animal lay quite flaccid. At this stage, on testing the sciatic nerves with the faradic current no muscular contraction took place, although the leg muscles contracted to a much weaker current applied directly. On the other hand the muscles at the place of injection were quite white and contracted very slightly. By next day the paralysis of the peripheral nerves had usually passed off and the tetanic condition had become reestablished. The pupils were fully dilated and the least touch caused a spasm. This condition might last for three or four days and then gradually passed off by the fifth or sixth day. The muscles regained their normal electric excitability in some cases but not in others, and in the latter event death followed. Larger doses caused rapid paralysis of the motor nerves and rigor mortis of the voluntary muscles, and death.

With doses of 1 to 5 mgms. there is very distinct increase of the spinal reflexes, but no symptoms indicating paralysis or paresis of the peripheral nerves. When the sciatic nerves were protected by tying the aorta low down and 2 mgms. cathinin sulphate dissolved in water given per aortam, there occurred at once great increase of reflexes and tetanic spasms in a few minutes.

*Cats.* With doses of 0.1 to 0.2 gram the animal was violently affected. In a few minutes the pupils dilated, salivation was profuse and there gradually came on symptoms of cerebral unrest. In an hour the restlessness, twitchings, increase of spinal reflexes, startings, and similar symptoms were extreme, and in two hours the animal was running round in circles and making sudden leaps and dashes, while apparently it also had hallucinations of hunting and catching mice. It was wildly excited and paid no attention to any obstacles, but banged up against them. Possibly the extreme dilatation of the pupils had something to do with this and prevented it seeing objects clearly. The spinal reflexes were increased, but the heart and respiration were not much affected. By the end of five hours most of the symptoms had passed off and it soon after seemed to have completely recovered.

Another large cat which got 0.4 gram cathinin sulphate subcutaneously showed similar symptoms to begin with, along with

vomiting and slight diarrhoea, but forty minutes after administration it had a series of violent tetanic spasms. Its respiration became very rapid and shallow and its legs paretic. Three hours after administration it had another series of very violent tetanic convulsions. In the intervals between them it lay on its side with fully dilated pupils and breathing very rapidly, and half an hour later it died in a convulsion. An examination was made at once when the motor nerves were found to be quite excitable to the faradic current, the heart was in diastole and all its chambers filled with dark venous blood, the small intestine was very empty and peristalsis was going on actively. Death had evidently resulted from asphyxia due to the tetanic spasm in which it died.

*Rabbits.* In rabbits the symptoms were much less severe. A rabbit (2180 grams) received 0.2 gram subcutaneously, and in fifteen minutes the pupils were dilated and there was a tendency to tremor and stiffness in the limbs. During the next hour these increased and some degree of paresis were added. In two hours the paresis was more marked, but there was at the same time spasm, rigidity, and tremor in the legs. The heart and respiration remained unaffected. The symptoms gradually passed off in about five hours.

Cathinin therefore in frogs acts from the beginning as an excitant of the brain and spinal cord. No stage of somnolence or narcosis is apparent. It paralyses the peripheral nerves when given in sufficient dose, but the paralysis passes off in a comparatively short time. Like cathin it is a muscle poison, the poisoning beginning very soon at the place of injection, and only slowly passing to more distant muscles. Small doses excite the brain and cord without markedly affecting the voluntary muscles or the peripheral nerves. In cats it produces violent cerebral excitement and a great or less increase of spinal reflexes. The peripheral nerves are paralysed to a certain degree only. The peristaltic movements of the intestinal canal are increased. In rabbits the symptoms are of the same kind but much less severe.

## ACTION OF CATHIDIN

Cathidin is not of great interest pharmacologically. As it is insoluble in water and does not form soluble salts it is difficult to administer, and I had to give it to frogs in as small quantities of 40 per cent alcohol as would dissolve each dose.

*Frogs.* Doses of 0.0025 to 0.005 gram were given hypodermically in this way, and as soon as the effects of the alcohol had passed off (which was very soon) the pupils dilated and there was a slight increase of reflexes lasting for some days. With 0.1 the increase of reflexes was much greater (almost to tetanus) and the muscles were more or less deeply poisoned at the site of the injection of the alkaloid. When 5 mgms. were given *per os* in alcohol the only effect was a slight increase of reflexes lasting for three or four days.

*Rabbits.* Doses of 0.2 to 0.5 gram dissolved in alcohol and injected subcutaneously in small rabbits had no effect beyond what might be expected from the amount of alcohol given.

*Cats.* A cat (1800 grams) had 0.5 grams cathidin mixed with water put into its stomach by means of a tube. For some hours there was no apparent effect and then the pupils dilated and remained so till next day. A similar amount given subcutaneously dissolved in alcohol caused vomiting the same day and dilated pupils next day, otherwise no effects were noticed.

## ACTION OF THE LEAVES ON MAN

*Man.* When the leaves are chewed continuously during a forenoon there is vaguely felt a certain degree of stimulation. Ten grams can be chewed in half an hour without causing any very definite sensations. An infusion made with 10 to 20 grams of finely powdered leaves and drunk at once was sometimes followed by a feeling of fulness in the head. Probably the stimulant and refreshing effect of small amounts is only fully appreciated by those accustomed to the drug, just as one finds in the case of tobacco and tea, where habitual indulgence leads to a certain craving and unrest which are assuaged by the next dose in a way

quite inappreciable to those not accustomed to a particular stimulant-narcotic.

It is possible that a fourth alkaloid or a decomposition product is obtainable from kat. As I have stated in my other paper Mr. D. B. Dott sent me a small quantity (0.17 gram) of the pure sulphate of an alkaloid which he found in the washings from preparing cathidin. One milligram caused violent tetanus in frogs, 2 mgms. paralysed the sciatic nerves and voluntary muscles in thirty minutes and the animal did not recover. I had not enough material to carry out a satisfactory series of experiments.

All these alkaloids, while differing in the details of their pharmacological action, show a close generic resemblance to each other, and in this respect are analogous to the various active principles present in such drugs as opium, coca leaves, and cinchona bark. Their actions fully explain the use of kat as a stimulant-narcotic. They are more closely allied in action to cocain and caffen than, say, to morphin or nicotin. They have no apparent action on sensory nerves or on the sensorium. Like cocain, caffen, and benzoyl-ecgonin, they exert a marked influence on voluntary muscles. I have elsewhere expressed the opinion that the stimulant effect of these substances is exerted not only on the nervous system but on the muscular system,<sup>4</sup> and although exact experiments are wanting to confirm this, my observations on the action of the catha alkaloids lead me to believe that it is correct.

<sup>4</sup> The Coca Alkaloids, British Medical Journal, i, 1889.



# THE CHEMICAL AND PHARMACOLOGICAL PROPERTIES OF HEDERIN, A SAPO-GLUCOSIDE CONTAINED IN THE LEAVES OF COMMON IVY (HEDERA HELIX)

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In a previous paper dealing with the properties of mowrin,<sup>2</sup> a glucoside of mowrah seeds, the word sapo-glucoside was introduced to designate members of a sub-class of the glucosides with similar properties to saponin in preference to calling them saponins since it is always confusing to apply the name of an individual to a class group.

In this paper it was pointed out that sapo-glucosides possessed many similar properties to bile salts, such as lowering surface tension and as a result concentrating upon a surface or interface; haemolysing red blood corpuscles at high dilution frothing and giving other properties of soap solutions; possessing a bitter taste of long persistence; giving a purple coloration with strong sulphuric acid alone, similar or identical with that given by bile salts and furfurol called Pettenköfer's test, or by  $\alpha$ -naphtol and a carbohydrate and the strong sulphuric acid in Molisch's test; causing on intravenous injection a slowing of the heart-beat and lowering of blood-pressure. The colour test above referred to with strong sulphuric acid only, shows that the sapo-glucoside molecule contains both an aromatic nucleus of a type resembling  $\alpha$ -naphtol and also a carbohydrate nucleus capable of yielding furfurol with strong sulphuric acid. The aromatic nucleus prob-

<sup>1</sup> Research students in Bio-chemical Laboratory University of Liverpool.

<sup>2</sup> Bio-chemical Journal, vol. v, p. 94, 1910.

ably varies somewhat in the different members of the group as the test is found to vary somewhat in color and greatly in delicacy in different individuals. Thus, both mowrin and hederin show a deep purple with the greatest ease, indistinguishable from the Pettenköfer's test with bile salts and sulphuric acid, while ordinary saponin gives a red colour which only transiently gives a purple on heating.

On account of the interest attaching to this group it was determined to investigate the properties of another member to see in how far the peculiar chemical and pharmacological properties persisted throughout the group. For this purpose the saponogluco-side found in the leaf of the common ivy was selected on account of its easy accessibility to us and also because, so far as we could determine from the literature as available to us, its pharmacological properties have not hitherto been investigated. As no name other than that of "hederaglykosid" has hitherto been given to this substance we propose that it should be called "hederin" and use that name for it throughout this paper.

The properties of this glucoside are very variously described in the earlier literature, and this according to van Rijn is to be ascribed to the great experimental difficulties attending the separation of a pure product from the leaves.<sup>3</sup>

The method of preparation as described in van Rijn is as follows: The ivy leaves are washed and then minced and extracted with hot 90 per cent alcohol, and the extract is decolourised with charcoal. The alcohol is then distilled off and the residue dissolved in a small quantity of absolute alcohol and concentrated until the glucoside begins to crystallise out. The crystals are separated by filtration through a hot funnel, and are washed with cold alcohol and then with acetone, after which the substance is dried. In this way there is obtained a fairly pure product which crystallises in beautiful colourless needles having a melting point of 233°C.

<sup>3</sup> J. J. L. von Rijn, *Die Glykoside*, Gebrüde Bornträger, Berlin, 1900, S. 332. The literature there cited is as follows: Kingzettl, *Pharm. Journ. and Transact.*, iii ser. vol. viii, p. 205; Hartsen, *Arch. d. Pharm.*, iii, R., Bd. vi, S. 299; Venet. *Bull. de l. Soc. Chim. T.* xxxiv, p. 23; Block, *Arch. f. Pharm.*, 1888, S. 962.

The substance is insoluble in water, chloroform, petroleum ether, slightly soluble in cold alcohol, more soluble in hot acetone, benzene, and ether, but the best solvent of all is hot alcohol.

After hydrolysing the substance with dilute sulphuric acid, the resulting solution reduces Fehling's solution. Alcoholic solutions of the glucoside are optically active having a specific rotation of  $[\alpha]_D^{22} = -47.5$ . The body is soluble in warm alkali and gives in the cold with concentrated sulphuric acid a beautiful violet colour. The water-free glucoside has the formula  $C_{32}H_{52}O_{10}$  and crystallises with two molecules of water of crystallisation, one of which is given off at  $100^\circ\text{C}$ . and the other at  $150^\circ\text{C}$ . By hydrolysing with 4 per cent sulphuric acid the glucoside yields glucose and a crystalline body of the formula  $C_{26}H_{40}O_4$



It is not hydrolysed by diastase. The hydrolytic product other than the sugar melts at  $287^\circ\text{C}$ . and gives on warming a beautiful orange yellow colour. On fusion with alkali formic acid is formed and also a body which gives with ferric chloride a pale violet colour. It also gives a crystalline acetyl derivative

#### CHEMICAL PREPARATION AND PROPERTIES

As we did not find the method of preparation given by van Rijn to be quite satisfactory we had recourse to the following procedure:—

The freshly gathered ivy leaves were freed from their stalks, chopped up into small pieces, thoroughly washed with warm water and pressed from adherent moisture. The residue was now extracted several times with 95 per cent alcohol at the boiling point and filtered through a hot funnel. The dark green solution so obtained was freed from chlorophyll by repeated treatment with animal charcoal. The solution finally obtained was faintly yellow and contained only a trace of chlorophyll. This solution was carefully concentrated and continuously scratched with the idea of inducing crystallisation. We were however unable on any occasion to obtain crystals. As the solution was concentrated the glucoside showed its colloidal nature by sepa-

rating out as a gelatinous mass. At this stage water was added in excess to complete the separation, the precipitate filtered through a Büchner funnel and washed thoroughly with warm water. The substance so obtained after drying on porous plates was dissolved in the smallest possible quantity of hot absolute alcohol and attempts were made to crystallise, but again proved unsuccessful. Recourse was now had to precipitation with ether, it having been found that by this means we obtained a finer and less gelatinous precipitate than that given by water precipitation. This process was repeated until a perfectly white amorphous substance was obtained.

Preparations made from different samples of ivy leaves by the above method gave concordant analytical results and had the same melting point as the crystals of pure substance (*vide infra*) so that it was assumed that the preparations, though not crystalline, were pure.

We found that the physical properties of the glucoside were practically the same as those described by van Rijn. It was easily soluble in hot alcohol, glacial acetic acid, acetone, and practically insoluble in water, ether, chloroform, benzene, and all the usual organic solvents. It was found that the purple colour given by concentrated sulphuric acid was practically the same as that given by mowrin and this reaction proved a very delicate test for the presence of the glucoside. Although it was found impossible to crystallise the body in quantity, beautiful needles were obtainable on a microscope slide as follows: A small quantity of substance was placed on a clean dry slide and a drop of acetone and absolute alcohol added covered with a slip and allowed to stand until crystals appeared; this usually taking about an hour. On one occasion a sufficient quantity of crystals were obtained with which to do a melting point. This was found to be  $231.5^{\circ}\text{C}$ . (uncorrected). The appearance of these crystals is shown in figure 1.

Many combinations of solvents, together with the usual aids to crystallisation, such as scratching, seeding, cooling, etc., were tried in order to obtain a quantity of substance in the crystalline condition but all proved unsuccessful.

*Chemical Composition.* Qualitative analysis shewed that the substance contained no nitrogen and was also free from inorganic matter.

After drying at 150°C. a combustion was made with the following results:

0.1598 gram gave 0.3759 gram  $H_2O$  = 8.802 per cent H  
0.3759 gram  $CO_2$  = 64.154 per cent C  
by difference 27.044 per cent O

The above figures are consistent with the formula given by van Rijn ( $C_{32}H_{52}O_{10}$ )

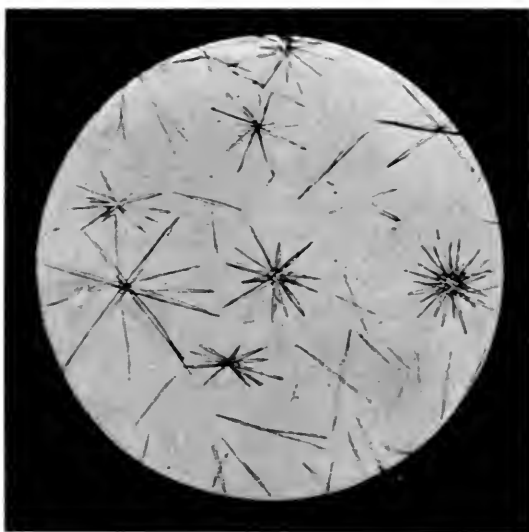


FIG. 1. DRAWING OF MICROSCOPIC CRYSTALS OF HEDERIN

#### MOLECULAR WEIGHT BY PHYSICAL METHODS

Attempts were made to determine the molecular weight by the usual physical methods, with unsatisfactory results. The freezing-point method was tried first, but it was found that hederin *raised* the freezing point of the solvent—glacial acetic acid. The boiling-point in alcoholic solution was then determined but it was found that the molecular weight increased with

the concentration. The raising of the freezing point of a solvent is usually assumed to be due to the formation of solid solutions. It is interesting to note that the same phenomenon was observed in the case of the glucoside mowrin previously investigated.

With reference to the attempts to determine the molecular weight by Beckman's method the following figures are interesting:

<i>Grms. of alcohol</i>	<i>Grms. hederin</i>	<i>Molecular weight</i>
25,945	0.077	142
25,945	0.261	475

In view of the extreme difficulty of inducing hederin to crystallise in quantity these results are extremely interesting. As will be noticed from the figures given, the molecular weight increases with the concentration shewing that association of molecules probably takes place; if this be so it is obvious that in a saturated solution the glucoside will separate out in the amorphous form. The fact that hederin crystallises easily on a cover-slip preparation may be explained as follows: the preparation consists essentially of a very thin film of fluid in which diffusion is reduced to a minimum and a relatively large crystallising surface is afforded. It is thus possible for single crystals to be deposited without fear of aggregation. The readiness of aggregation and ease with which jelly-like solutions form on cooling from alcoholic solutions show the tendency of the substance to become a colloid. It does however slowly diffuse through parchment paper when dissolved in dilute alkali.

*Products of hydrolysis.* Various methods of hydrolysis were tried, it was thought at first that glacial acetic acid would be the most suitable for the purpose owing to the ease with which hederin dissolves in it. This proved, however, to be unsatisfactory as is shewn by the fact that in one experiment in which a solution of hederin in this acid was kept boiling continuously for a period of seventy-two hours, only a small portion was hydrolysed. Both sulphuric and hydrochloric acids were tried; but in no case were we able to hydrolyse the glucoside completely without destroying it. Even autoclaving with 25 per cent sulphuric acid at 120°C. for many hours failed. This is not a

case of reversible reaction because, as will be shown later, one of the products of hydrolysis escapes.

Prolonged boiling with 25 per cent sulphuric acid was employed. When the hydrolysis was wanted for quantitative estimation of resulting products, the unchanged hederin was filtered off, dried and weighed. Later on, emulsin was tried but this also did not hydrolyse the glucoside with any degree of completeness.

The presence of a reducing substance in the products after hydrolysis having been shown by reduction of Fehling's solution, the nature of this substance was examined. It was found to be glucose, as it yielded an osazone which shewed the typical crystalline structure under the microscope. Glucose was given by hydrolysis with either acids or emulsin. In one case, however, after hydrolysis with hydrochloric acid, removal of the acids by distillation in vacuo, and subsequent extraction of the residue with methyl alcohol, an osazone was obtained which resembled somewhat that of galactosazone. Only a small quantity was obtained and was not further investigated; it was probably due to the formation of intermediate products.

By acid hydrolysis, furfural was obtained, being easily recognised by its odour and the formation of phloroglucide with phloroglucinol.

Quantitative experiments on the products of hydrolysis isolated, glucose and furfural were made but no concordant results could be obtained. It is interesting to note that furfural was not obtained by hydrolysing with emulsin. It is hence probable that the furfural is the degradation product of some carbohydrate present. A spectroscopic examination of the beautiful purple colour given by strong sulphuric acid was made with interesting results. Two absorption bands, one occupying the positions (590–610) and the other strongest at 525 and fading off towards the red end towards the violet, were observed. The band nearer *D* proved rather elusive and was usually only obtained after standing for several hours, and on further standing disappears. Some comparisons were made with this spectrum and that given by Pettenkøfer's test for bile salts. Although the two spectra are not identical the band nearer *D* is common to both

spectra, and this band is in fact the characteristic band because the other bands can be obtained without the presence of furfural, with cholic acid and sulphuric acid alone. As the Pettenköfer test requires furfural it is interesting to note that hederin can replace cane sugar (the source of furfural) in this test thus proving indirectly the presence of furfural, either as a constituent or decomposition product, in hederin.

#### HAEMOLYTIC PROPERTIES

Hederin possesses an intense haemolytic activity. Although in its natural condition in the leaf it behaves like an organic acid insoluble in water, yet water with which the leaves have been washed froths on shaking and this water although it only contains the merest trace, even when made isotonic by adding common salt, causes laking in a suspension of red blood corpuscles. The hederin when required for haemolytic experiments is dissolved in a small amount of decinormal alkali in which it is easily soluble, and diluted to the various strengths to be tested with normal saline or Ringer's fluid. The red blood corpuscles used were those of the sheep washed twice with normal saline, and then used as usual in such experiments in a 5 per cent emulsion in saline. The time given in the table shews the period necessary for complete haemolysis.

It is noteworthy that the peculiar effect first noticed by McLean and Hutchinson in a research in this laboratory and called by them the "haemolytic paradox" comes out clearly in the case of hederin solutions. That is to say it is not by any means the strongest solutions of hederin which cause haemolysis most rapidly, but comparatively dilute solutions. Thus, its optimum concentration, or that which haemolysed most rapidly, was found to lie at 0.3125 to 0.625 mgm. of hederin in 5 cc. or 0.006 to 0.012 per cent. A solution of this concentration laked the corpuscles practically instantaneously, either a stronger or a weaker solution than this laked them much more slowly.

This interesting property probably depends upon some colloidal aggregation of the saponin at this particular con-



centration as shewn by the results given in determining boiling point of dilute solutions above. The result is very striking with both mowrin and hederin, and quite astonishes the observer when first obtained. The first conclusion he arrives at is that he has accidentally made some mistake in carrying out the dilutions, but repetition shows that it is a true phenomenon. The effect is not obtained with ordinary saponin.

*Haemolysis by hederin. Part I. Strength of hederin solution = 0.25 per cent*

FIVE PER CENT EMULSION OF SHEEP'S ERYTHROCYTES	hederin solution	SALINE TO 5 CC.	WEIGHT OF GLUCOSIDE PRESENT IN 5 CC.	TIME OF COMPLETE HAEMOLYSIS
cc.	cc.	cc.	mgms.	
1	4	nil	10	Over 1 hour
1	2	2	5	Over 1 hour
1	1	3	2.5	12.5 minutes
1	0.5	3.5	1.25	6.5 minutes
1	0.25	3.75	0.625	3 minutes

*Part II. Same Solution diluted 32 fold*

1	4	nil	0.312	Immediate.
1	2	2	0.156	2 minutes
1	1	3	0.073	9.5 minutes.
1	0.5	3.5	0.036	{ Not complete after 1 hour.
1	0.25	3.75	0.018	

This paradoxical effect appears no matter how often the corpuscles are washed. The times of haemolysis with the stronger solutions vary considerably in an apparently accidental fashion as if the physical condition of the colloidal sapo-glucoside varied from one making up to another, but they are invariably longer than the more dilute solutions.

#### PHARMACOLOGICAL PROPERTIES

Hederin appears to possess all the well-marked pharmacological properties of the class of the sapo-glucosides. In the intact animal it is comparatively innocuous when given by the mouth as was shown to hold in the case of mowrin, but like that body the bitterness of the substance prevents the leaves

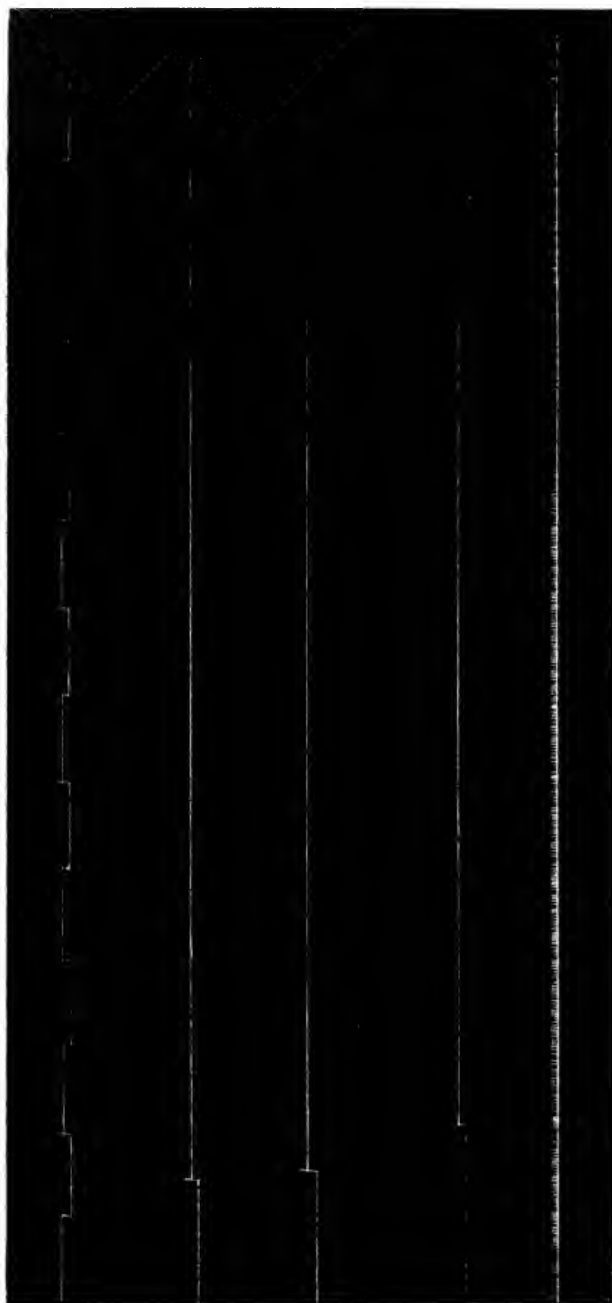


Fig. 2. CONSTRICTING ACTION ON PERIPHERAL VESSELS OF PITTED FROG

Caused by perfusion of 1 in 100,000 of hederin in Ringer's fluid through aorta. Bottom tracing, time in seconds. Top tracing, normal reversals of Schäfer tilter (2.5 cc. outflow capacity), when perfusing with Ringer's fluid alone. Tracings 2, 3 and 4, show reversals when 1 in 100,000 hederin is perfused.

being eaten by cattle. The powdered substance is a powerful irritant to the mucous membranes of nose and throat as was likewise noted for the case of mowrin. When injected intravenously in rodents it poisons in small doses, its death being produced chiefly by respiratory paralysis. For example, 0.5 to 0.75 cc. of a 2 per cent solution injected either subcutaneously or intravenously will kill a rat of 130 to 150 grams within a few hours. Before death laboured and slow breathing are observed and a paralysis of the hind limbs sets in. Examination afterwards shows the blood partially laked, and the heart in an asphyxial condition. The intestinal area appears to be specially affected as in the case of all these sapo-glucosides. The intesti-



FIG. 3. RATE OF FLOW THROUGH PERIPHERAL VESSELS OF FROG  
Dilating action of 1 in 1,000,000 in Ringer's solution.

nal vessels are engorged and capillary rupture and bleeding into the intestine are observable. The urine in the bladder also contains haemoglobin.

*Effects on peripheral blood-vessels.* This was determined by perfusion through a pithed frog in the method introduced by Schäfer. At a concentration of 1 in 100,000 in Ringer's solution the drug shews a marked constriction but at 1 in 1,000,000 in Ringer this is changed into a strong dilatation. This is illustrated by the two tracings given below. In fig. 2. is shown the constricting action of the 1 in 1,000,000 solution, in fig. 3 the dilation effect of 1 in 1,000,000 is seen.

*Effects on general blood-pressure.* Injection of 1 to 2 cc. of a 1 per cent solution in an anaesthetized mammal causes a marked

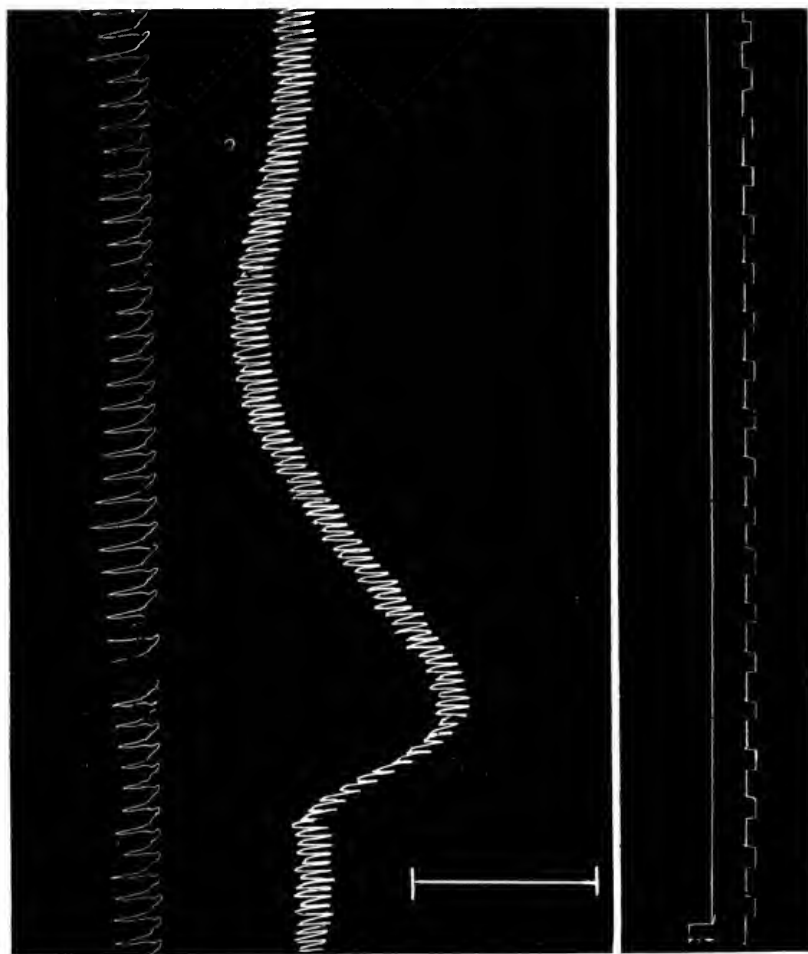


FIG. 4. EFFECTS ON ARTERIAL BLOOD PRESSURE OF INTRAVENOUS INJECTION OF HEDERIN  
 Dog of about 6 kgs. anaesthetized with chloroform and ether. One cc. of 1 per cent solution of hederin injected into internal saphenous vein. Top line, respiration by Marey tambour connected to tracheal cannula. Second line, carotid B. P. mercurial manometer. At break, 100 mm. of B. P. cut out to save space. Vertical line on left shows 50 mm. of B. P. Bottom lines, signal and zero B. P., and time in seconds.

fall in blood-pressure. This demonstrates that when in equilibrium with the blood and tissues, the minimal or dilatation effect produced by the smaller amount in Ringer (viz., 1 in a million) is the result obtained. In other words the proteins and fats form a reservoir for the hederin and protect the small vessels.

*Effects on the heart.* The effects of hederin on the perfused heart are the same as those found in the case of mowrin, namely, slowing the beat with increased tonus. The effects are shown on the accompanying tracing (fig. 5).

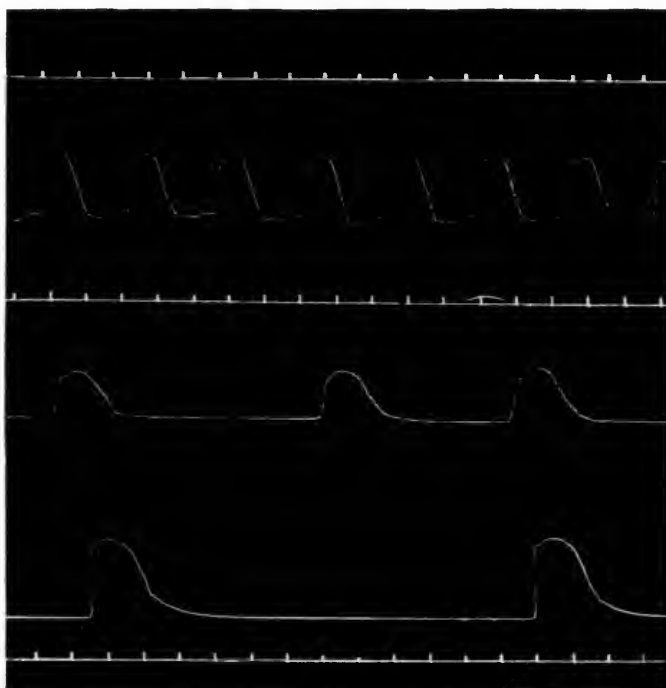
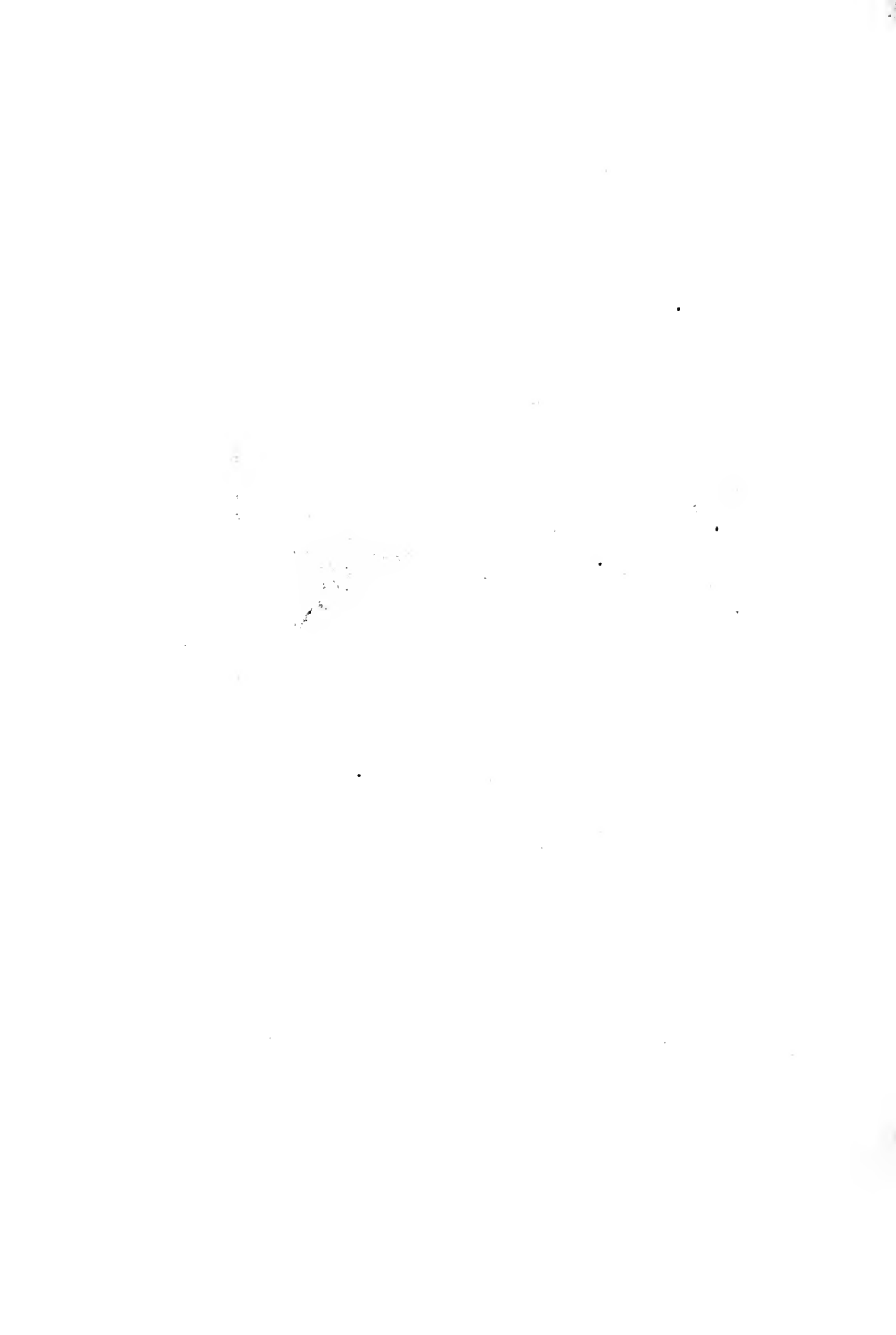


FIG. 5. EFFECTS OF HEDERIN IN SLOWING HEART-BEAT

Upper tracing normal. Second tracing five minutes, and third tracing ten minutes after injection of 1 cc. of 1 per cent solution. Pithed frog, heart *in situ*, injection by hypodermic needle into liver.



## EPINEPHRINE

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In 1901 Strehl and Weiss<sup>1</sup> published results to show that the flow of epinephrine from the glands to the blood was continuous. By clamping off the adrenal vein on one side after the gland on the other side had been removed, the blood pressure fell immediately and continued to fall as long as the circulation was interrupted. When the clamp was removed the blood pressure quickly reached its normal level. Complete removal of the adrenals was soon followed by low blood pressure and death.

Assuming this report to be true, one of us (McG.) in 1908, working under the direction of Professor Gottlieb, attempted to demonstrate a difference in the epinephrine content of the blood of rabbits before and after adrenalectomy, using the uterine strip method as an indicator. It was thought that if the fall of the blood pressure were due to a lack of the epinephrine in the blood, after some time all of the substance would be exhausted and a difference could be shown. For example, if the normal blood diluted fifty or seventy-five times would show a distinct epinephrine effect on the uterine strip, then after the removal of the glands when the pressure had fallen to one-half the normal or one-fourth the normal height, a stronger concentration of the blood would be necessary to get an effect comparable with the effect produced by normal blood. This would be so if the fall in pressure were due to the lack of the epinephrine in the blood, providing also that the uterine strip was excited only by the epinephrine. Our attempt was a failure. No positive difference could be detected before and after adrenalectomy. More recently O'Con-

<sup>1</sup> Strehl and Weiss, *Archiv für die gesammte physiologie*, 1901, lxxxvi, p. 107.

nor<sup>2</sup> working in the same laboratory also failed to show such a difference by using the same method, but he discovered apparently, the reason for the failure. According to him, blood on clotting liberates something that has an epinephrine-like action on the uterine strip, therefore the method is inadequate to show such a difference, even though such existed. In our own work we have found that slight changes in the reaction, the addition of traces of oxidizing reagents such as potassium permanganate, and many ions have an influence not only on the uterine strip but also on the reaction of the whole organism to epinephrine. By using the perfusion method of Lawen and Trendelenburg, O'Connor was not able to show the presence of epinephrine in the peripheral blood of normal animals. This agrees with the conclusion previously reported by Stewart.<sup>3</sup> In more recent work however Stewart<sup>4</sup> casts considerable doubt on the idea that pressor principles develop on clotting.

If one of the functions of the gland be to aid directly in sustaining the blood pressure, we should expect by the use of an adequate method to find some indication of epinephrine in the peripheral blood and, theoretically at least, the work of Strehl and Weiss would be correct. Our work, however, on dogs, rabbits and cats gives no support to their claims. Young and Lehman<sup>5</sup> and more recently Young<sup>6</sup> alone, Kahn,<sup>7</sup> and also Hoskins and McClure,<sup>8</sup> have all reported work in which they failed to find evidence in support of the conclusions of Strehl and Weiss.

While O'Connor found no epinephrine in the peripheral blood, he did find it in the adrenal vein. The amount was decreased after section of the splanchnics. Stewart<sup>9</sup> found it in the adrenal

<sup>2</sup> O'Connor, *Archiv. für experimentelle pathologie und pharmacologie*, 1912, lxvii, p. 195.

<sup>3</sup> Stewart, *Journal of Experimental Medicine*, 1911, xiv, p. 337.

<sup>4</sup> Stewart, *Journal of Experimental Medicine*, 1912, xv, p. 547.

<sup>5</sup> Young and Lehman, *Journal of Physiologie*, 1908, xxxvii, p. liv.

<sup>6</sup> Young, *Vincent Ergebnisse der Physiologie*, 1910, ix, p. 509.

<sup>7</sup> Kahn, *Archiv für die gesammte physiologie*, 1911, xli, p. 209.

<sup>8</sup> Hoskins and McClure, *American Journal of Physiologie*, 1912, xxx, p. 192. Also, *Archives of Internal Medicine*, 1912, x, p. 343.

<sup>9</sup> Stewart, *Journal of Experimental Medicine*, 1912, xv, p. 205.



vein only after stimulation of the splanchnics. These observations confirm the work of Dreyer<sup>10</sup> who first demonstrated the secretory nerves to the gland and also the work of Cannon and la Paz,<sup>11</sup> Biedl<sup>12</sup> and Langlois<sup>13</sup> and that of Cybulski<sup>14</sup> who first showed that epinephrine was a normal constituent of the blood.

The continuous secretion of epinephrine into the blood and its influence under some conditions on the blood pressure has been shown in a much simpler way than any of the above methods, by Popielski.<sup>15</sup> By pressure on the vena cava just internal to the entrance of the adrenal veins, the secretion of the gland is dammed back and it accumulates in the glands and vessels. The immediate effect of the pressure is a great rise of blood pressure. If this be maintained for a short time and then suddenly released, the pressure quickly drops to normal and the returning circulation sweeps the stored epinephrine into the blood stream and a typical epinephrine effect results. The same, though smaller, effect was obtained by direct massage of the gland. Massage of other organs was without this influence and negative results were obtained after removal of the adrenals.

An examination of the tracings will show that the results obtained by this method are quite different from the tracings presented by Strehl and Weiss in support of their claims. Their results may be attributed to vasomotor reflexes arising either in the walls of the compressed vessels or the immediate region. The work of Latschenberger and Deahna<sup>16</sup> would support this opinion. In fact, they give tracings, very similar to those presented by Strehl and Weiss, but which Latschenberger and Deahna present as the result of stimulation of afferent nerves. Zuntz<sup>17</sup> would explain this last result as being due directly to a local asphyxia.

<sup>10</sup> Dreyer, *American Journal of Physiology*, 1899, ii, p. 203.

<sup>11</sup> Cannon and la Paz, *American Journal of Physiology*, 1911, xxviii, p. 64.

<sup>12</sup> Biedl, *Archiv für die gesammte physiologie*, 1897, lxvii, p. 443.

<sup>13</sup> Langlois, *Revue Scientifique*, 1897, p. 303.

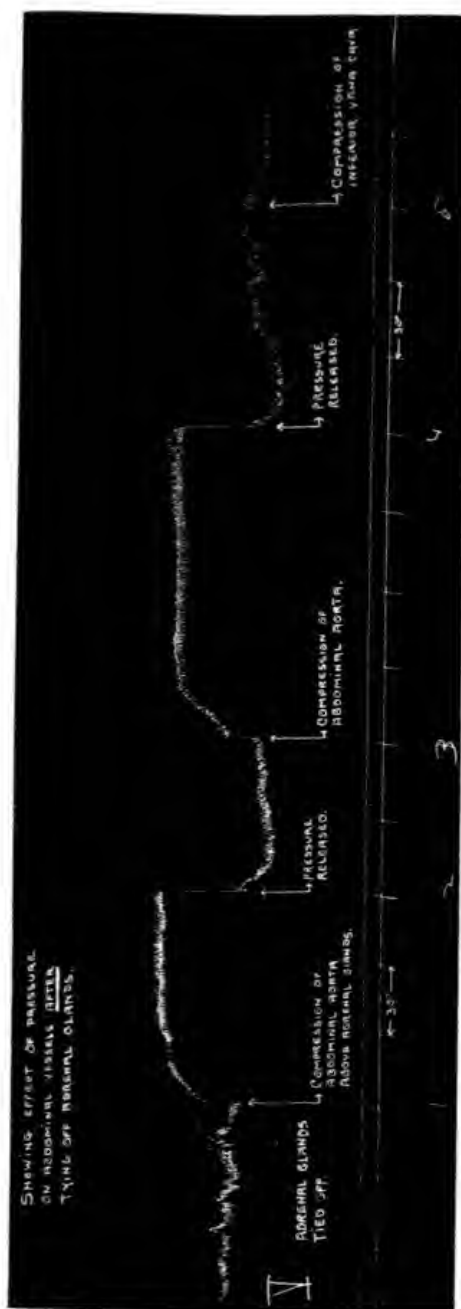
<sup>14</sup> Cybulski, *Centralblatt für physiologie*, 1895, p. 172.

<sup>15</sup> Popielski, *Archiv für die gesammte physiologie*, 1911, cxxxix, p. 571.

<sup>16</sup> Latschenberger and Deahna, *Archiv für die gesammte physiologie*, 1876, xii, p. 157.

<sup>17</sup> Zuntz, *Archiv für die gesammte physiologie*, 1878, xvii, p. 404.





TRACING No. II

This is of interest in connection with the relation of asphyxia and the adrenals to glycosuria.

The transient effect of epinephrine on the blood pressure is in accord with the results of Stewart, O'Connor and others, and, taken together with the result of the removal of the glands, indicates that the pressure influence is only one of the several functions of the glands. The fall of pressure after the removal of the glands cannot be due so much to the direct loss of the pressure substance, as to the loss of some other less known body or bodies. The presence of more than one functioning body in the glands is also indicated by the difference in the action of the drug when administered hypodermically and intravenously both in rabbits and also in human asthmatic patients. In one case the blood pressure symptoms are predominant while in the others changes in the metabolism and respiration are the functions most influenced. It is possible that these actions may be due to different oxidation products of the same substance. Ransom<sup>18</sup> however believes that the pressor and glycosuric powers are so situated in the molecule that one may be lost while the other is retained. He states that the glycosuric power is the first to be lost when potato tryosinase is allowed to act on epinephrine.

In an attempt to determine which of the properties is first lost by oxidation, we boiled the substance for varying lengths of time in dilute alkali or allowed the action to take place more slowly by standing at room temperature, and then determined the change in the glycosuric and blood pressure action. Our results agree with those of Ransom, the glycosuric power being more strongly reduced than the pressure raising power. This is directly opposite to the results we had anticipated.

The methods for determining these two properties are not equally sensitive. The pressure effects can be obtained by very small amounts while the amount necessary to cause glycosuria is very much larger. If the volume necessary to cause glycosuria in a rabbit is 0.5 cc. of 1 : 1000 solution, we may expect blood pressure effects with one-fiftieth of this amount; consequently when

<sup>18</sup> Ransom, Proceedings of the Royal Society of Medicine, xix. p. 25.

we get pressure effects after boiling to make sure that the glycosuric powers are equally influenced, we must inject enough of the dilute solution hypodermically, to correspond to the blood pressure power of the normal drug necessary to cause the glycosuria. To illustrate: If it requires 1 cc. of the normal drug to cause glycosuria a dilution of fifty times this will cause pressure symptoms. After boiling, the undiluted drug may be reduced to correspond to a dilution of fifty times, and it will still show a distinct influence on the blood pressure, but the glycosuric power, if reduced a corresponding amount, will manifest itself only when we inject 50 cc. If it be more influenced than the pressure principle, then more than 50 cc. will be required.

A criticism of the method is the difficulty of recognizing changes in the sugar metabolism with anything near the delicacy or accuracy that changes in the blood pressure may be detected. The normal animal can conceal large temporary changes in the glycolytic activities, while the blood pressure is exceedingly responsive to slight variations in the pressure raising body. For these reasons and also for the reason that sugar sometimes appears in the urine of rabbits very easily, conclusions as to the relative rate of the oxidation of these bodies have been drawn from very positive and unfailing results. Since, at most, we have found only a slight diuretic effect from samples devoid of pressure influence, it is quite certain that the glycosuric power is lost at least as early as the pressure influence and when the pressure influence is still present but much reduced, large amounts of the drug failed to cause glycosuria, we conclude that the glycosuric property is most easily destroyed by the action of dilute alkalies. The following protocols warrant this conclusion:

I. Rabbits were used for the glycosuric test. Each was injected hypodermically with adrenalin (Parke, Davis and Company, solution 1 : 1000, of 0.9 per cent NaCl, freshly prepared). One cubic centimeter of any of the preparations used, always caused glycosuria in the controls.

No.	Weight in grams	
1	2000	Control
2	2000	Control
3	2250	Solution boiled one minute with an equal volume $\frac{N}{10}$ NaOH and neutralized before use
4	2000	Solution boiled one minute with an equal volume $\frac{N}{10}$ NaOH and neutralized before use
5	2250*	Solution boiled two minutes with an equal volume $\frac{N}{10}$ NaOH and neutralized before use
6	1450	Solution boiled two minutes with an equal volume $\frac{N}{10}$ NaOH and neutralized before use
7	1720	Solution boiled two minutes with an equal volume $\frac{N}{10}$ NaOH and neutralized before use
8	625	Solution boiled three minutes with an equal volume $\frac{N}{10}$ NaOH and neutralized before use.
9	1020	Solution boiled three minutes with an equal volume $\frac{N}{10}$ NaOH and neutralized before use
10	1120	Solution boiled three minutes with an equal volume $\frac{N}{10}$ NaOH and neutralized before use
11	1980	Solution boiled for four minutes with an equal volume $\frac{N}{10}$ NaOH and neutralized before use
12	2020	Solution boiled for four minutes with an equal volume $\frac{N}{10}$ NaOH and neutralized before use
13	1150	Solution boiled for four minutes with an equal volume $\frac{N}{10}$ NaOH
14	2500	Solution boiled for five minutes with an equal volume $\frac{N}{10}$ NaOH
15	2000	Solution boiled for five minutes with an equal volume $\frac{N}{10}$ NaOH
16	2000	Solution boiled for five minutes with an equal volume $\frac{N}{10}$ NaOH

Result on the blood pressure. This was determined on dogs. After boiling for one minute, the pressure effect was reduced one-half. After five minutes, the strength was 1:50,000. That boiled for two, three, and four minutes, showed little difference from that boiled for five minutes. Different commercial preparations are not destroyed with equal rapidity.

*Glycosuric effects.* The control animals and those receiving an injection of epinephrine which was boiled for one minute excreted large

amounts of sugar in the urine. None of the others showed any sugar, though in all the urine was thin and pale, and apparently increased in volume. How far this points toward a glycosuria we cannot say; the only positive statement that can be made is that after the pressure principle had been almost destroyed the glycosuric effect was also lost. The diuretic effect showed that some of the glycosuric influence still existed, and we think this is in ratio with the loss of pressure effect, as some pressure influence still persisted. Similar results were obtained from slow deterioration of the epinephrine at room temperature.

The above experiment clearly illustrates that, when the blood pressure is almost destroyed by oxidation, the glycosuric effect of small doses is totally destroyed. It might be possible, however, still to produce glycosuria by using larger doses. To answer this objection, the following experiments were performed, using supracapsulin:

II. Three control rabbits, weighing 2190 grams, 1980 grams, 2170 grams, were each given 1 cc. of the supracapsulin, 1 : 1000, hypodermically. In ninety minutes, all showed sugar in the urine (heavy tests).

The supracapsulin was then boiled for ten minutes, with an equal volume of  $\frac{N}{10}$  NaOH. At the end of this time, it still showed considerable pressure powers.

Four rabbits weighing 1730 grams, 1540 grams, 1670 grams, 1520 grams, were each given an equivalent of 3 cc. of the original solution of supracapsulin. No sugar occurred in the urine during the next eighteen hours, although an average of 52 cc. each of urine was passed (35 to 68 cc.). The urine was pale and clear. In this, when considerable pressor effect still persisted, three times the amount normally required to produce glycosuria failed to do so. This is in keeping with the statement of Ransom, but might be due to the fact that the animals easily shows a change in pressure, while glycosuric changes are not so easily shown. The character of the urine indicates a diuretic influence, which may be commensurate with the loss of blood pressure effect.

III. One cubic centimeter of  $\frac{N}{5}$  NaOH was added to 10 cc. of supracapsulin. After standing for six days the blood pressure was not reduced more than one-half; yet 2 cc. of this injected into a rabbit hypodermically caused at most a trace of sugar in the urine.

IV. A solution of adrenalin was prepared from the powder. This was boiled with 1 cc. of  $\frac{N}{1}$  NaOH and neutralized with HCl. The final

volume was 1 : 1000. The blood raising power was reduced to one-fourth of its original strength. Four cubic centimeters of this given to a rabbit by hypodermic injection caused no glycosuria.

V. In like manner 12 cc. of supracapsulin was boiled ten minutes with 2 cc. of saturated sodium bicarbonate and neutralized. The final volume was 25 cc. The blood pressure effect corresponded to between 1 cc. and 1.25 cc. of a 1 : 20,000 solution of the original solution; 21 cc. of this solution was injected into a rabbit weighing 2100 grams. This had a pressure power of at least 0.85 cc. 1 : 1000 solution. There was no symptom of glycosuria. The volume of urine in fifteen hours was 10 cc. There was no indication of sugar in it.

These and other experiments support the opinion that the glycosuric action of epinephrine is more rapidly destroyed than the pressure action. Another noticeable feature is the loss of toxicity by boiling. There was no depression, no deaths and much less change in the respiration after the hypodermic injections of large volumes of the boiled drug than after the injection of an equivalent blood pressure raising volume of the unboiled. This indicates a parallelism between the glycosuric and the toxic action of the drug.

Attention has been called repeatedly to the depression which frequently follows the rise in blood pressure from epinephrine. Van Leersum,<sup>19</sup> Hunt,<sup>20</sup> Lohman<sup>21</sup> and Weidlein<sup>22</sup> especially have worked on this phenomenon. The cause has been attributed to cholin bases and other impurities. It is concluded that the secondary depression is not a characteristic of the pure epinephrine base, and since cholin has been prepared from the residue of the gland after the epinephrine had been removed, it has been singled out as the most prominent cause. It is formed on standing and for this reason old preparations are said to cause greater secondary depression than freshly prepared solutions. It may be due to decomposition of the pressor principle.

<sup>19</sup> Van Leersum, *Archiv für die gesammte physiologie*, 1907, cxviii, p. 215.

<sup>20</sup> Hunt, *American Journal of Physiology*, III, p. xviii and V, p. vi, *Proceedings*.

<sup>21</sup> Lohman, *Archiv für die gesammte physiologie*, 1907, cxviii p. 215.

<sup>22</sup> Weidlein, *Journal of Industrial and Engineering Chemistry*, 1912, iv, p. 640.



The action of ions on uterine strips suggested to us that these might play a part in modifying the action of epinephrine on the whole animal. It is known that commercial preparations have acids and other bodies added to preserve them. Depressant bodies may develop on standing but these seem to be of less importance than the reaction of the preparation and the condition of the animal.

One cubic centimeter doses of acids or alkalies,  $\frac{N}{10}$  have a very slight influence on the blood pressure of a dog when injected intravenously. If, however, acid is given with epinephrine, or a short time before it, there is usually a secondary depression where none would have occurred without it. Alkali, in like manner, counteracts the secondary depression. Ammonium hydrate is more effective than sodium hydrate in this respect. Repeated injections of epinephrine often cause a secondary depression when a single dose is ineffective. This is probably due to the formation of fatigue products. More important than the reaction of the drug is the condition or individuality of the animal. We have found that in some animals adrenalin may always cause a marked secondary depression while the same solution in another animal will never cause a depression, even with repeated doses, and with the addition of acid.

The secondary fall is perhaps only a symptom of more important changes in the organism. The nervous system is profoundly influenced, and the symptoms resemble a mixture of stimulation and depression. The animals appear depressed and are apparently paralysed with larger doses. At the same time, they are much more susceptible to the action of strychnine<sup>23</sup> than normal animals. This influence on the nerve is probably secondary and due to products formed in the muscle, as the direct application of the drug to the nerve is not known to produce this effect.

We have no good method of determining the influence of the muscle on the nerve, but it must be important. While the stimulation of the nerve influences the muscle, the action must be reversible. This also holds good for other nerve poisons, as is indicated

<sup>23</sup> Mostrom and McGuigan, *Journal of Pharmacology and Experimental Therapeutics*, 1912, iii, p. 521.



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by the well known fact that the high blood pressure during a strychnine spasm remains elevated much longer in a curarized than in a noncurarized animal.<sup>24</sup> So also the profound changes in the sugar metabolism which follows the injection of epinephrine cannot be without influence on the nerve centres, and must aid in the secondary depression.

Our experiments on the secondary depression have been few in number, but they clearly indicate that there are several factors which may coöperate as causes. Among these, are the reaction of the drug, the products formed in the muscles as a result of the action, and above all the condition, or individuality, of the animal. The relative importance of these will be discussed in a later paper.

#### CONCLUSIONS

The results of other investigators in so far as they deal with changes in the epinephrine content of the blood have been confirmed.

There is nothing to indicate that the rapid fall of blood pressure which may follow clamping of the adrenal veins is due to a decreased epinephrine content of the blood; though by a proper method (Popielski) a continuous secretion of the adrenal glands, and the transient effect of this under some conditions on the blood pressure, can be demonstrated.

Evidence is presented to show that the glands exert more than one function and that the substances essential for the maintenance of these functions are oxidized with different velocities. A relationship between the glycosuric and the toxic properties is indicated.

Several factors may operate to cause the secondary fall of blood pressure which follows the intravenous injection of epinephrine, among these are, the reaction of the drug, the products formed as the result of previous injections, and especially the condition or individuality of the animal.

<sup>24</sup> Cushny, Text book of Pharmacology and Therapeutics, 1910, p. 205.



## THE PERIPHERAL ACTION OF CERTAIN DRUGS WITH SPECIAL REFERENCE TO THE LUNGS

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*Methods.* In a number of the experiments described below I have used a method for studying bronchial changes which I believe has not been described before. It consists in opening the chest of a spinal animal in the medium line after which a specially designed metal plate (which I may refer to as a shield, fig. 1) is fitted into the chest in such a manner as to form a partition between the lung and the heart. A notch in the shield passes around the pedicle of the lung which is thus left freely movable in the cavity formed by the chest wall on the outer side and the metal shield on the inner side. At the diaphragm the shield is bent outward at a right angle and thus forms a protecting floor for the cavity and prevents any movement of the diaphragm or abdominal viscera from altering the air space in the cavity. The edges of the shield are sewed to the edge of the chest wall and the chest is then closed by stitching together the tissues at the edges of the divided sternum. If desirable the chest may be tied rigidly to the operating board, but with nonconvulsive poisons this is unnecessary in spinal animals. A bent tube is passed through the chest wall into the cavity which can thus be connected to a large tambour or Brodie bellows by a rubber tube. The whole

<sup>1</sup> Certain portions of this work were presented in a paper read before the St. Louis Medical Society, November 9, 1912, and were abstracted in the Bulletin of the St. Louis Medical Society. Later observations which are also included in this article were presented before the Indiana Academy of Science at Indianapolis, November 29, 1912, while certain other features were described before the American Physiological Society at Cleveland, December 31, 1912 (abstracted in the American Journal of Physiology).

lung is thus left entirely free and in a condition which should very closely approximate the normal. Artificial respiration has been used in all the experiments. In all cases the brain and medulla of the animal have been destroyed by the injection of chloroform into the right vertebral artery, as has been described in another paper.<sup>2</sup> In many cases, however, I have used either the same

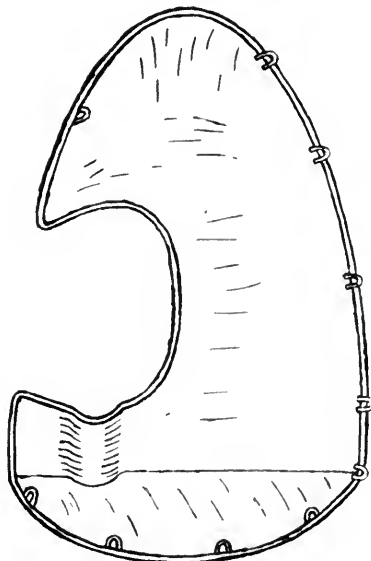


FIG. 1. LUNG SHIELD FOR THE RIGHT LUNG

The shield is fitted into the chest between the pericardium and the right lung. The pedicle of the lung passes through the large notch shown on the left side of the drawing. At the lower edge of this notch is a groove in the shield into which the inferior vena cava fits. The shield is stitched to the chest wall and the thorax is closed. A tube inserted into the outer wall of the chest is connected by tubing to the recording tambour. For medium sized dogs the greatest length of the shield is 13 cm. the greatest width 10 cm.

method<sup>3</sup> which I employed in a former series of experiments on the bronchial musculature or else I have used a modification of this method which consists in simply passing a metal tube of

<sup>2</sup> Jackson, D. E., *Journal of Pharmacology and Experimental Therapeutics*, 1912, iv, p. 33.

<sup>3</sup> Jackson, D. E., *Journal of Pharmacology and Experimental Therapeutics*, 1912, iv, p. 59.

about  $\frac{1}{4}$  inch diameter transversely through the anterior part of the animal's chest in about the fourth or fifth interspaces and about  $\frac{3}{4}$  inch outward from the edge of the sternum on either side. Both ends of the tube are open and to one is attached a rubber tube leading to the recording instrument, while on the other end a tube is placed which connects with a large metal drum (about 4 inches in diameter), having the heads made of readily distensible rubber dam. This drum serves to accommodate the excess air which is driven out of the chest by the expanding lungs and which is usually too great in amount for the capacity of the recording tambour. The amount of air passing in and out of the large drum is regulated by a screw clip on the rubber tube connecting the drum to the metal tube through the chest. Air from the chest cavity enters the metal tube by way of a large number of small holes bored into the tube along that portion of its length which is enclosed within the chest cavity. For nonconvulsive poisons I have found this method very satisfactory and the easiest of all to carry out. In addition to these methods I have also frequently used various forms of plethysmographs for both the upper and the lower lobes of the right lung and for the entire lung, my object being to test out various observations in as many ways as possible. My main object in these experiments has been to determine the action of a number of substances upon the bronchioles. While this work is by no means complete, it has perhaps served to throw some light upon certain general features which may be more fully developed later on.

*The relation of broncho-dilatation to other phenomena.* It was shown by Januschke and Pollak<sup>4</sup> in 1911 that intravenous injections of epinephrine produced a dilatation of the bronchioles if these had been previously constricted by muscarine. This observation has since been corroborated in different ways by Trendelenburg,<sup>5</sup> Jackson<sup>6</sup> and Park.<sup>7</sup> Notwithstanding the fact

<sup>4</sup> Januschke, H., und Pollak, L., Arch. f. exper. Pathol. u. Pharm., 1911, lxvi, p. 205.

<sup>5</sup> Trendelenburg, Zent. f. Physiol., 1912, xxvi, p. 1.

<sup>6</sup> Jackson, D. E., Journal of Pharmacology and Experimental Therapeutics, 1912, iv, p. 59.

<sup>7</sup> Park, Journal of Experimental Medicine, 1912, xvi, p. 55S.

that all these results appeared to be in agreement I have still felt that certain features of this work should be tested out in other directions. And this opinion has been greatly strengthened in the light of further observations which I have made more recently. The particular point which I have had in mind is the relation which the marked rise in general blood pressure produced by epinephrine may have to the dilation of the previously contracted bronchioles. Januschke and Pollak attempted to clear up the matter by injecting into the animal a quantity of ergotoxine sufficient to paralyze the vasoconstrictor nerve endings after which they administered muscarine and when the bronchioles had contracted they observed that injections of epinephrine caused a dilatation although no rise in general blood pressure occurred. Conversely I have shown in another place that a great increase in the general blood pressure, such as may be produced by clamping the abdominal aorta, does not dilate the bronchioles when they are tonically contracted by pilocarpine. There are certain objections which might be raised against both of these observations and while I believe that both conclusions are entirely correct, I have thought that the following experiment ought to be described in connection with the above mentioned observations.

*Equalization of blood pressure.* The object of this experiment is to show that the dilatation of the bronchioles does occur after epinephrine in a perfectly normal manner when the general blood pressure is kept at a constant level. The procedure is as follows: A spinal animal is prepared as above described and arranged for pulmonary and blood pressure records. The femoral veins are connected to burettes for injecting the drug. The femoral arteries (and the left carotid also if desired) are picked up and large cannulas with side tubes are connected to the vessels, the cannulas opening toward the heart. The bulldog clips are left on the arteries for the time being. The side tubes of the cannulas are then connected to the straight ends of a T-tube, the side tube of which is connected to a rubber tube about  $\frac{1}{4}$  inch in diameter and about 3 feet long. A glass T-tube is placed in the middle of this rubber tube in such a manner that the side neck of the T-tube can be connected to the side tube of a mercury manometer. The



distal end of the 3-foot rubber tube is then passed upward and the end is finally turned down into a beaker. The straight tubes of the cannulas in the arteries are connected in the one case to an injecting burette and in the other to a small piece of tubing closed by a clip. From the burette the whole system of tubes are filled with normal salt solution. A little salt solution should be placed in the beaker in order to render the siphon-system active at the beginning of the experiment. It is probably better to have both femoral arteries and one carotid connected to the siphon tube, but for ordinary cases the two femorals are sufficient. When these preparations are complete a quantity of hirudin in salt solution is injected into the femoral vein. It is desirable for an average sized dog to use at least 100 mg. The injection is best made in small repeated doses, as large injections lower the blood pressure rather markedly. The corresponding levels of the mercury in the two manometers are now made the same by adjusting the height of the beaker above the animal and the bulldog clamps are removed from the femoral arteries. The mercury in both manometers oscillates correspondingly. A little epinephrine is injected and as the arterioles constrict, the excess blood in the aortic system passes over to the beaker. As the arterioles dilate, the blood siphons back into the dog. Some provision should be made for keeping the beaker and tubes warm.

When the apparatus is completely adjusted and the respiratory tracing is being regularly recorded, a small injection of pilocarpine ( $\frac{1}{2}$  to  $1\frac{1}{2}$  mg.) is given. As soon as the bronchial constriction is well marked an injection of epinephrine is made. As soon as the drug is carried around, the bronchioles begin promptly to dilate. The blood can be seen to pour rapidly over into the beaker. If the vascular constriction is sudden and great, there may be a slight rise in general pressure at the beginning of the action of the epinephrine. If, however, large mouthed cannulas be connected with three arteries (one carotid and both femorals) this may be entirely avoided.<sup>8</sup> It is better to use these peripheral arteries

<sup>8</sup> It is possible that this method for equalizing the blood pressure may have been used before, but I have found no reference to it in the literature. I feel fairly certain that it has not been used for the purpose to which I have here applied it.

than to subject the animal to a severe operation in order to place a large cannula into a big vessel such as the abdominal aorta. For every effort should be made to keep the animal in the most nearly normal condition possible if one hopes to obtain thoroughly satisfactory lung tracings. The bronchial dilatation produced by the epinephrine is thus seen to be of short duration (fig. 2) and the pilocarpine constriction again soon comes on. A second injection of epinephrine, however, again causes a dilatation. From this it appears that the bronchial dilation is independent of the systemic rise in blood pressure. But this experiment does not show that changes in the pulmonary circulation may not influence the bronchioles and I have considered the feasibility of connecting one of the above described equalizing systems to a pulmonary arterial branch in order to keep down changes in the pulmonary pressure. I do not believe, however, that such procedure would show any change in the results as already secured, for the variations in pulmonary pressure in the living animal are always small and the operation itself would by no means add to the success of the experiment. After death it appears that rigor mortis does not come on nearly so soon in an animal which has been injected with hirudin as in a normal animal. And without making any careful observations it has seemed to me that the anticoagulating action of hirudin lasts longer in a spinal animal than in one whose brain and medulla are intact.

Incidentally it may be mentioned that the method which I have here used to carry out the internal perfusion of the lungs may quite well be extended under certain conditions to the study of other organs, such as the spleen, kidney, intestinal loop, hind limb, etc., when these organs are enclosed in appropriate oncometers. Reactions occurring within the organs themselves may be studied by sectioning the entering nerves or painting them with cocaine or phenol.

*Abnormal conditions.* At this place I should like to mention a few things which have appeared either to others or to myself to be possible causes of abnormal reactions within the lungs. In a series of perfusion experiments on isolated heart-lung preparations

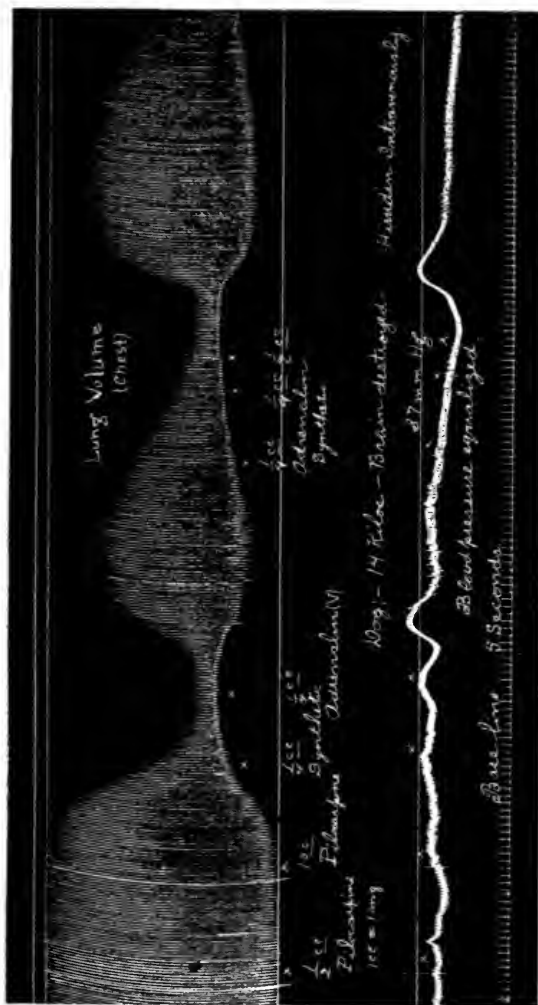


FIG. 2. LUNG VOLUME AND BLOOD PRESSURE TRACINGS FROM A SPINAL DOG

By means of the equalizing apparatus described in the text, the blood pressure was prevented from rising to a great height. Pilocarpine was injected and caused a marked broncho-constriction. Synthetic adrenalin was then injected and caused a dilatation which soon passed off. Later injections of adrenalin then caused a more marked dilatation. The slight variations in blood pressure were mostly due to the small size of the necks of the cannulas connected to the femoral arteries to equalize the pressure which otherwise would have risen to a point considerably above the top of the lung volume tracing. This shows that the dilating action of epinephrine on the bronchioles is independent of the rise in general blood pressure. The dog had received 100 mg. of hirudin.

Evans<sup>9</sup> has recently noted that "in many cases the lung vessels after a few minutes' perfusion, constricted to such an extent that the blood flow practically ceased. Attempts were made to discover the cause of this phenomenon, but with no great success. It was not due to the influence of an alteration of temperature, or to changes in the reaction of the blood, to coagulation, to products formed in defibrination of the blood, to air emboli, to insufficient or too great pressure, nor to the hirudin solution employed." He ventures "to think that it was often due to a too perfect removal of carbon dioxide from the blood, since in some cases the administration of 10 per cent carbon dioxide removed the resistance almost at once." In this connection it is interesting to recall that (Einthoven<sup>10</sup>), Dixon and Brodie,<sup>11</sup> and Trendelenburg<sup>12</sup> have found that carbon dioxide produces a constriction of the bronchioles<sup>13</sup> which is of apparently both central and peripheral origin. With reference to temperature Hess<sup>14</sup> has reported experiments indicating that the application of cold to the surface of the lungs may decrease the pulmonary circulation by probably more than 30 per cent.

I feel quite sure that I have met with instances in which various ones of these factors must have figured, but there are certainly several other more or less obscure causes which may coöperate to influence pulmonary reactions as obtained under ordinary experimental conditions. Some of these will be discussed later in this article. Perhaps I should mention here the exhaustion of the animal.

It is the usual occurrence that as the animal becomes exhausted its pulmonary reactions become less and less sensitive. A pecu-

<sup>9</sup> Evans, C. L., *Journal of Physiol.*, 1912, xlv, p. 225.

<sup>10</sup> Einthoven, *Pflüger's Archiv*, 1892, li, p. 423.

<sup>11</sup> Dixon and Brodie, *Journal of Physiology*, 1903, xxix, p. 166.

<sup>12</sup> Trendelenburg, *Zent. f. Physiol.*, 1912, xxvi, p. 1.

<sup>13</sup> I have not had an opportunity to carry out any special experiments with carbon dioxide, but in view of the action of this substance on the walls of the blood-vessels, it has seemed not improbable to me that carbon dioxide may really dilate the bronchioles and that there may possibly have been some error (temperature?) in these earlier observations.

<sup>14</sup> Hess, R., *Deutsche Arch. f. Klin. Med.*, 1912, Bd. 106, H. 5-6, p. 489.

liar stiffness or slowness of collapse and inflation seems to come on to the lung tissues. Nearly all drugs may cease to show visible changes in the pulmonary tracing and no further observations can be made. I suspect there are several causes for this, and that these may vary considerably in different experiments. Cold undoubtedly must be considered. In plethysmographic methods this is especially liable to occur. The sensitiveness and reponse of the parietal and visceral pleura must be taken into account. With most forms of plethysmographs it has seemed to me that disturbances of the blood or lymph vessels of the lungs may occur. The process of inflating the lungs by positive pressure through the trachea is certainly very far from normal. I have repeatedly considered the practicability of inflating the lungs by exhaustion methods and hope to do this in a series of future experiments. Dixon and Brodie have already emphasized the importance of the depressing action of ether or chloroform vapor on the endings of the bronchial nerves. It has often seemed to me that the constant forcing of comparatively dry air into the trachea must be very trying on the delicate lung structures, for artificial heating and moistening devices do not probably by any means replace the organs which naturally carry out these processes. In my experiments I have, however, tried to keep the air both moistened and warmed. Concerning the action of the body metabolites (both normal and abnormal) upon the bronchial musculature, we must say but little. There is another condition which I had observed many times before I fully appreciated its significance. If one injects certain substances such as vanadium into an animal in considerable quantity there will after a time be produced a sort of continuous tonic contraction of the bronchioles. If the force of the air blown into the trachea is sufficient to still inflate the lungs, then one may fail to get any further responses of the bronchioles to any drugs. It was partly because of this phenomenon that I was slightly misled in a former series of observations.<sup>15</sup> It was there observed that if an "animal be treated with atropine until pilocarpine is no longer able to produce any

<sup>15</sup> Journal of Pharmacology and Experimental Therapeutics, 1912, iv, p. 10.

bronchial constriction, then the administration of vanadium will still induce a clearly demonstrable constriction of the bronchioles. In this instance however, the later injection of adrenalin fails to cause any dilatation." The observation as stated is substantially correct, but from it the inference might be drawn that atropine paralyzes the broncho-dilator nerve endings. It is more probable, however, that the reason why I obtained no dilatation in that series of observations when epinephrine was injected after the preceding series of drugs, was that in the absence of any action on the broncho-constrictor nerve endings the production of a broncho-constriction by vanadium required doses sufficient to set up a rather marked muscular tonus which the epinephrine could not overcome. But it is also probable that both atropine and vanadium, under experimental conditions at least, exercise a rather distinct depressing influence on the broncho-dilator nerve endings. I probably ought to mention that this opposing action of vanadium toward the later action of epinephrine is apparently very general, for after large repeated doses of vanadium have been injected into an animal, then the injection of epinephrine in any sized doses is almost without any influence at all as far as raising the general blood pressure is concerned. This is apparently due to the gradual establishment of a very great and prolonged tonic contraction of the muscular walls of all the arterioles of the body. This is also borne out by oncometric observations of the kidney, spleen, etc., but a part of the result may be due to a gradual weakening of the heart. And it seems probable that in large doses vanadium also exercises some depressing action on probably all those nerve endings which are normally stimulated by epinephrine. With respect to the action of atropine on the bronchioles, I may say that small doses do not appear to affect the dilator endings, while the constrictor endings are readily paralyzed. A further action which I have seen a good many times in animals which had previously received sufficient quantities of atropine to paralyze the constrictor endings is a slight contraction of the bronchioles when later injections of atropine were given. I believe this is due to a slight direct action on the bronchial musculature and that it probably bears no relation whatever to the nerve supply. In

perfused kidneys I have also observed that atropine causes a vascular constriction when administered in rather large doses. The action on the bronchioles is probably of the same nature. It seems that large or repeated doses of atropine also tend to weaken or paralyze the broncho-dilator nerve endings. It is peculiarly difficult, however, to prove this point, for in order to show a dilator action on the lungs it is necessary to first have some broncho-constriction present. But when doses of atropine sufficient to completely paralyze the constrictor nerve endings are given, then it is no longer possible to secure the initial broncho-constriction for the dilator test by means of pilocarpine, muscarine, etc. We are then compelled to give such substances as vanadium, etc., which act on the bronchial muscle fibers in order to secure a contraction. We are then confronted with the possibility that so strong a muscular tonus may be set up that the epinephrine may not be able to cause a dilation even though the dilator endings may not have been paralyzed and may be duly stimulated by the epinephrine. With care, however, the experiment can be carried out and it appears to be unquestionably true that the dilator nerve endings are not paralyzed but remain readily active after quantities of atropine, which are much more than sufficient to paralyze the broncho-constrictor nerve endings (fig. 3). Since these reactions may occur in a spinal animal in which both vagi have been cut, and in consideration of the action of epinephrine elsewhere, it seems impossible to avoid the conclusion that epinephrine acts by stimulating the broncho-dilator nerve endings. I emphasize this merely because of its bearing on observations to be discussed later.

*Post mortem dilatation.* At death the bronchioles generally slowly constrict. After a time they may dilate some again. I have observed that if epinephrine in considerable quantity is injected just as the heart stops, then if artificial respiration be kept up, a marked dilatation of the bronchioles may appear after a little while. I suspect this is due to the slow moving around of the epinephrine by the lung movements until finally the drug reaches the dilator nerve endings and still stimulates them to active dilatation.

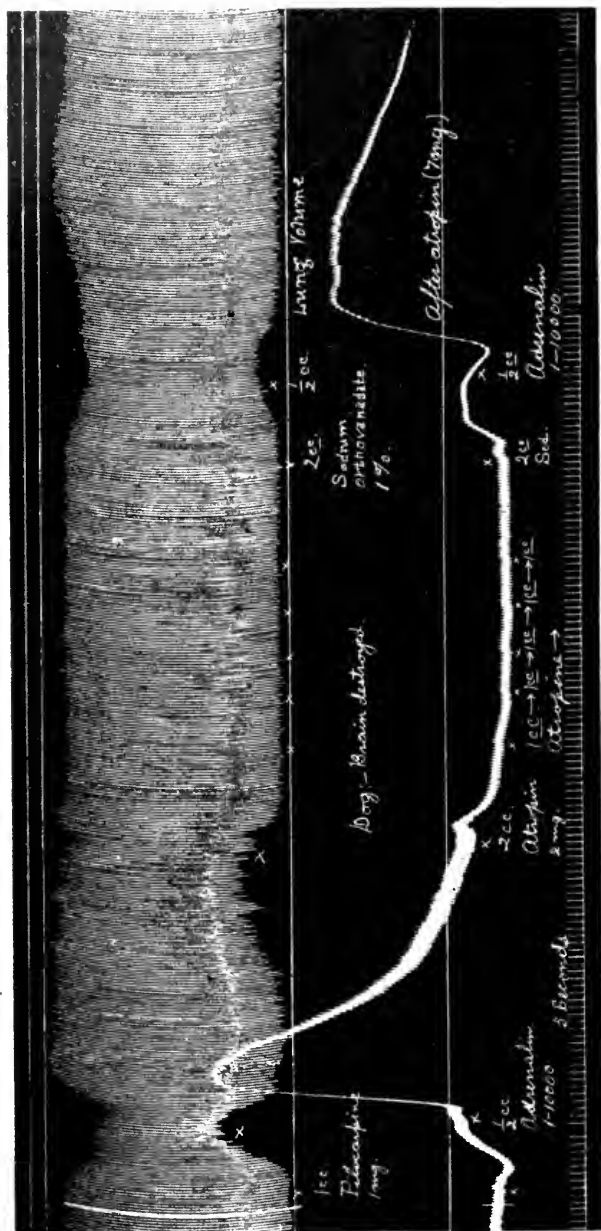


FIG. 3. LUNG VOLUME AND BLOOD PRESSURE, SPINAL DOG

At the left of the tracing pilocarpine was injected and produced a broncho-constriction. Adrenalin was then injected and produced a temporary dilatation. As the pilocarpine constriction returned, atropine was injected and produced a dilatation. Later more atropine was injected (7 mg. in all). (1 or 2 mg. of atropine would paralyze the broncho-constrictor nerve endings.) Finally 2 cc. of 1 per cent sodium-orthovanadate solution (neutralized with HCl) was injected and produced a contraction of the bronchioles from direct muscular stimulation. Adrenalin then again produced a dilatation. This shows that quantities of atropine sufficient to paralyze other structures on which the drug acts specifically does not paralyze the broncho-dilator nerve endings. But very large doses of atropine seem to weaken these endings appreciably. A direct muscular action by the atropine may be partly responsible for this later action.



## GROUP REACTIONS

*Paradoxical dilatation.* If one carefully prepares a fresh spinal animal for recording lung volume changes and then injects a small quantity ( $\frac{1}{2}$  to  $\frac{3}{4}$  mg.) of pilocarpine, there generally will be observed a slight brief initial contraction of the bronchioles followed by a slight but very distinct and rather prolonged dilatation (fig. 4). This applies particularly to the spinal dog with both vago-sympathetic nerves sectioned low in the cervical region. If then one prepares another fresh spinal animal in a similar manner, but also ties off the adrenal glands, then upon injection of the pilocarpine, the initial constriction appears, but the later dilatation is extremely slight or wholly absent.

In an admirable series of experiments it has been recently shown by Dale and Laidlaw<sup>16</sup> that the injection of pilocarpine into a fresh animal leads to a very considerable increase in the secretion of the adrenal glands. By tests made largely upon the pupil and nictitating membrane under a variety of varying conditions they have apparently proved this point beyond any question. The results of their experiments at once throw light on the probable explanation of the paradoxical dilatation of the bronchioles under small doses of pilocarpine. Cushny, Elliott, Langley, Anderson, Bayliss and perhaps a good many other observers have already noted in some form or other, similar paradoxical reactions in other organs of the body. If this is the correct explanation of the secondary dilatation of the bronchioles, as I feel reasonably sure it is, mention should be made of the fact that under pilocarpine we have two opposite and opposing forces acting on the bronchioles. The pilocarpine directly stimulates the constrictor nerve endings, while the epinephrine secreted stimulates the dilator nerve endings. (Possibly the pilocarpine may also exercise some stimulating action on the muscle fibers of the bronchioles.) It is obvious that the recorded result in any given case will be the algebraic sum of these two actions. When the dose of pilocarpine is small and the adrenals are well stored with epinephrine, we may generally look for the best

<sup>16</sup> Dale, H. H. and Laidlaw, P. P., *Journal of Physiology*, 1912, xlv, p. 1.

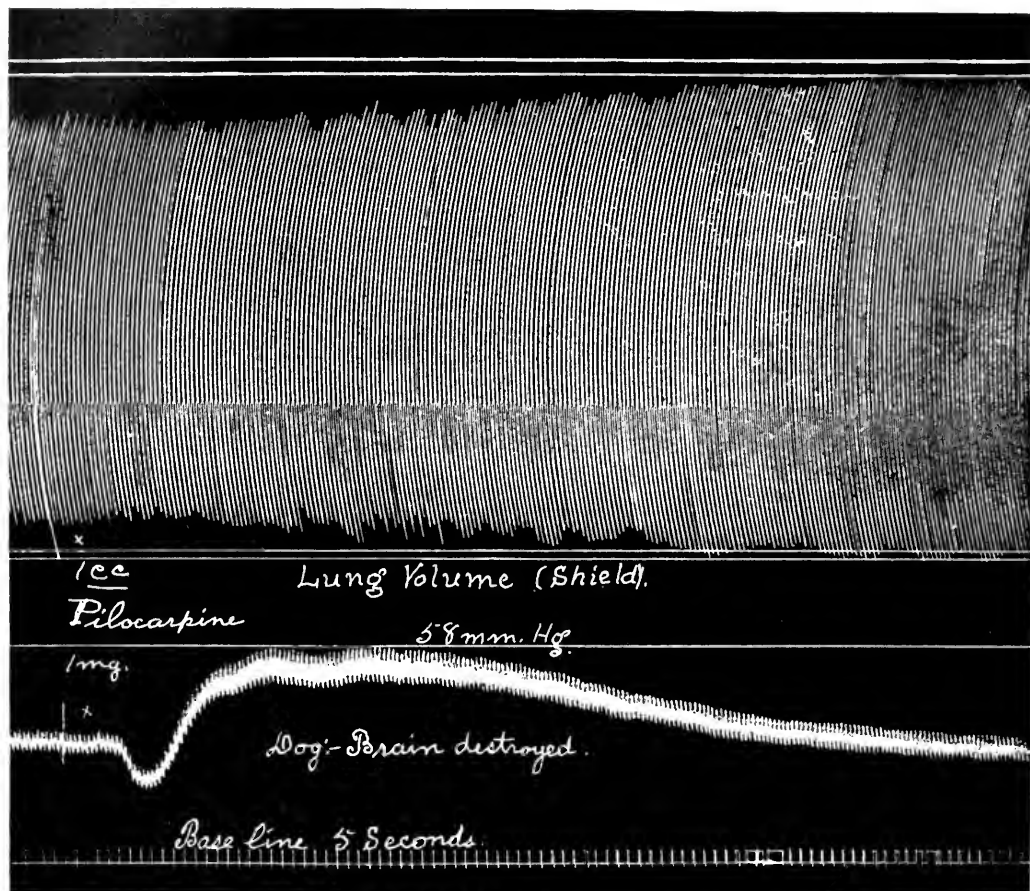


FIG. 4. LUNG VOLUME AND BLOOD PRESSURE TRACINGS FROM A SPINAL DOG

At the beginning of the tracing 1 mg. (1 cc.) of pilocarpine was injected into the femoral vein. The pressure falls slightly and the bronchioles constrict a little. But soon the pressure rises and shows two or three characteristic slight falls near the highest part of the tracing. At the same time the bronchioles begin to slowly dilate. This paradoxical action on the lung volume is rather prolonged.

results.<sup>17</sup> But it may happen then that the endings of the constrictor nerves are not very sensitive to the pilocarpine, so no initial constriction will be obtained. If then the bronchioles had been fully dilated previously, i.e., if no tonus had been present, then the epinephrine might not be able to produce any visible dilatation. But if on the other hand the constrictor nerve endings were very sensitive to the pilocarpine, then so marked a tonic constriction might be produced by the pilocarpine that the quantity of epinephrine secreted could not overcome the pilocarpine action.

It is interesting to note that long ago Dixon and Brodie<sup>18</sup> apparently noted a similar paradoxical dilatation of the bronchioles under muscarine. I have not so far had an opportunity to do any experiments with muscarine, but I feel sure that the delightful accuracy of their description of the action of this body as compared with pilocarpine could leave little to be gained by a repetition of their experiments. I am, however, inclined to believe that the dilatation may be better explained on other grounds than those chosen by them. These authors suspected that muscarine stimulated both the constrictor and the dilator nerve endings, and in case some initial tonus was present, the dilators might respond first. In opposition to this I have not seen any dilatation of the bronchioles produced in exhausted dogs by pilocarpine after sufficient doses of atropine to paralyze the constrictor endings had been injected. But the previous treatment in these cases might have depressed the sensitiveness of the dilator endings. They may, however, then respond quite well to epinephrine if a correct degree of tonus is first secured, and ligation of the adrenal glands appears to prevent the secondary

<sup>17</sup> It perhaps should be mentioned here that a number of observations are on record indicating that the injection of a toxic dose of serum into a sensitized guinea pig is also followed by a slight early lung dilation which later is succeeded by great broncho-constriction which leads to death. The preliminary dilatation here appears so similar to that following pilocarpine that it might seem worth while to consider whether or not acute anaphylactic shock (in a guinea pig) is also accompanied by the liberation of epinephrine. Cf. Auer, J., *American Journal of Physiology*, 1910, xxvi, p. 442.

<sup>18</sup> *Loc. cit.*, p. 155.

dilatation by pilocarpine. As noted by these authors, the secondary dilatation is often absent. The reason for this is obvious. But often the constriction produced by moderate doses of pilocarpine shows a peculiar more or less rhythmic form in which the bronchioles first constrict, then dilate a little, then constrict again, and again dilate a little, but in the long run the constriction each time is greater than the following dilatation, so that after a time a strong tonic contraction is produced. I suspect this rhythmic effect is due to the irregular control of the bronchioles which is exercised from second to second by the pilocarpine and by the epinephrine secreted. It is possible of course that pilocarpine may possess some action on some portion of the nervous dilator mechanism (ganglia, etc.),<sup>19</sup> but in *spinal dogs* whose vago-sympathetic nerves are sectioned low in the neck, it does not appear probable that such an action can play much part (cf. Möllgaard<sup>20</sup> who by degeneration methods has obtained evidence indicating that the pulmonary sympathetics have their ganglion cell stations in the middle cervical ganglion in the dog).

*Tonic constriction.* It was suggested above that vanadium would produce a marked tonic broncho-constriction when given in sufficient quantities. It seems quite evident that a large number of other substances may also have this action. Probably many salts of the metals act thus. Barium and bromine certainly do, but they are much less active than vanadium, and I suspect that several of the alkaloids such as morphine and quinine should be put in this class. The action of quinine is rather interesting, for in addition to producing a moderate broncho-constriction, which comes on rather rapidly after intravenous injection and is very prolonged in character, it seems that the rate of inflation and collapse of the lungs is rendered distinctly slower and more sluggish. Recovery from this condition is rather slow and the

<sup>19</sup> In at least two experiments I have obtained some evidence that the dog may have some sympathetic dilator nerve fibers passing to the lungs from the spinal cord by way of the stellate or inferior cervical ganglion. In these two cases stimulation of the upper cervical cord after section of the vago-sympathetic nerves produced a dilatation of the lungs.

<sup>20</sup> Möllgaard, H., Scand. Arch. f. Physiologie, 1912, xxvi, p. 315. (Abstract in Zent. f. Physiol., 1912, September, No. 12.)

action occurs after atropine as well as before. I suspect that  $\beta$ -iminazolyethylamine should be mentioned here although I have performed no experiments with this drug. Probably most of these substances act mainly, if not entirely, on the bronchial muscle. Whether or not epinephrine produces a dilatation after the more active of these substances, seems to be practically a question of the *degree of the tonic action*.

*Bronchial asthma.* It has seemed to me that a practical question of considerable importance might be briefly considered in connection with this subject. It was suggested by Dixon and Brodie that an attack of spasmodic bronchial asthma could scarcely occur if full paralysis of the broncho-constrictor nerve endings had been secured by atropine. Evidence has been accumulated in recent years which makes it seem, at least, possible that in many cases bronchial asthma may simply be a form of anaphylactic reaction. If in these cases it should happen to be the muscle fibers, as has been shown to be true in at least one instance by Auer,<sup>21</sup> rather than the myoneural junctions in the bronchioles which show the special sensitization, then in all probability a sufficient quantity of epinephrine would be more effective in warding off the attack than would atropine, although the latter drug might also be of advantage.

*Stimulation of adrenal nerve endings.* It is conceivable that a drug might directly stimulate the secretory endings of the nerves in the adrenal glands. In that case we should expect the first large dose of the substance, if it were very active, to cause a marked decrease in the readily available supply of active substance in the glands. I believe that I have tried out at least two drugs which should be placed in this group. I refer to ergotoxine<sup>22</sup> and tyramine. Injection of a small quantity of either of these substances into a fresh animal whose adrenal glands are intact, gives a brief dilation of the bronchioles if these had previously been slightly contracted. I have also suspected that isoamyl-

<sup>21</sup> Auer, J., *Journal of Experimental Medicine*, 1910, xii, pp. 151 to 175. Also *Proceedings of the Society for Experimental Biology and Medicine*, 1910, vii, p. 104.

<sup>22</sup> Jackson, D. E., *Loc. cit.*, p. 69.

amine might act in this way, but so far my experiments have not been very conclusive with this substance. One should always bear in mind in making these tests that a previous asphyxia during the anaesthetization, or fear, prolonged etherization, low blood pressure, etc., may have markedly depleted the store of epinephrine in the glands. Probably a number of other drugs including hydrastinine,<sup>23</sup> may act similarly.

*Stimulation of ganglia on adrenal nerves.* This action has already been demonstrated by a number of writers. The typical drug manifesting this action is nicotine. I have performed a good many experiments with this drug, but unfortunately the results have not been entirely satisfactory. I believe there can be no doubt at all that nicotine<sup>24</sup> causes a secretion of epinephrine in a fresh animal and that this epinephrine is carried to the lungs and regularly produces a dilator effect. I have observed this many times, but the action of nicotine on the bronchioles is undoubtedly more complicated than this. When the alkaloid is injected, the first change produced in the bronchioles is a slight, abrupt initial constriction. This is rapidly succeeded by the dilation. When the dilation, which may last for two or three minutes, passes off, the lungs may return apparently to their normal condition. (This applies, as do all other experiments described in this paper, to spinal dogs.) The cause of the sudden brief initial constriction is possibly to be sought in ganglionic stimulation. (But the location of these ganglia when the vagi nerves are sectioned in the cervical region, is an open question.) It generally appears to be of too sudden a character to impress one as being wholly muscular in origin. It may possibly be due to stimulation of the constrictor endings, but I hardly believe so. Nor do I believe that the following dilation is due to any ganglionic stimulation by the nicotine, for section of the vagi low down in the neck does not affect the reaction. It seems probable therefore that increased adrenal secretion may cause the dilatation. Thus far the action of the drug does appear to be unduly obscure,

<sup>23</sup> Dale and Laidlaw, *Loc. cit.*, p. 2.

<sup>24</sup> Cannon, Aub and Binger, *Journal of Pharmacology and Experimental Therapeutics*, 1912, iii, p. 379. Dale and Laidlaw, *Journal of Physiology*, 1912, xlv, p. 1.

but in a few instances, and some of them were particularly well defined and so far as I could determine were free from any experimental error, I have observed another action of the substance. After a complete paralysis of the vaso-constrictor nerve ganglia by several injections of nicotine, then a broncho-constriction was produced by the injection of pilocarpine. Unexpectedly, another dose of nicotine caused a slightly slow but very complete dilatation of the bronchi. When those results were obtained I had no doubt but that the dilatation as shown in the tracings represented exactly the action which the nicotine had in those instances. I was unable, however, to make any suggestion as to the manner in which the nicotine acted and I should not mention the results here at all were it not for the fact that Dale and Laidlaw have recorded a dilatation of the pupil under conditions which makes it seem very probable to me that the action of the nicotine was the same in each case. Those authors had destroyed the brain, medulla and spinal cord of the animal and had removed the suprarenals and one superior cervical ganglion, but probably the destruction of the brain and medulla of my animals by chloroform and the later complete ganglionic paralysis by nicotine reduced the animals in each instance to practically the same condition. It is to be expected that other alkaloids of the nicotine group may act similarly to that substance on the lungs.

*Central stimulation.* We should expect that some substances might be capable of producing a secretion of epinephrine by stimulation of the brain or cord which might in turn result in bronchial dilatation. I have not positively demonstrated this action by any substance, but I have suspected that caffeine probably acts thus, at least to some extent. Pal<sup>25</sup> who has described the dilator action of caffeine on the lungs believed the drug acted by direct stimulation of the broncho-dilator nerve endings. I had observed this action several months before Pal's paper appeared, but the dilatation was so slight in comparison with that of epinephrine that I had cast the observation aside. The caffeine dilation comes on very slowly and is of a prolonged

<sup>25</sup> Pal, D. J., Deutsche medizinische Wochenschrift, 1912, xxxviii, September 19, p. 1774.

character. In my experience it is even less in extent than that of urotropine which also produces some dilatation. Asphyxiating poisons such as the cyanides may probably produce some broncho-dilatation by a sort of indirect central stimulation of the adrenals.

*Cocaine.* I need say only a few words about this substance. In my experience it produced no marked effect either in the way of a dilatation or a constriction of the bronchioles. There was some indication that the dilator endings were a little more sensitive to epinephrine after the cocaine had been injected (followed by pilocarpine to cause an initial constriction). Possibly in an animal whose brain and medulla were intact cocaine might have some further action.

*Agaricin, camphoric acid and sodium iodide.* A few words may be said regarding these bodies. Both of the first two of these substances have been credited with power to depress or paralyze the secretory nerve endings in the sweat glands, while sodium iodide is claimed to paralyze the inhibitory endings in the heart. It seemed possible to me that they might exercise some such action on the bronchial nerve endings. I was especially interested in determining whether or not they could paralyze the dilator endings, since the origin of these nerves is probably analogous to that of the sweat nerves. Injections of sodium camphorate produced no effect at all so far as I could determine on the bronchial innervation. Agaricin is difficult to get into a satisfactory solution, but as nearly as I could judge of its action there was a slight depression or paresis of the broncho-constrictor nerve endings. Sodium iodide seemed to be inactive on the bronchial nerves.

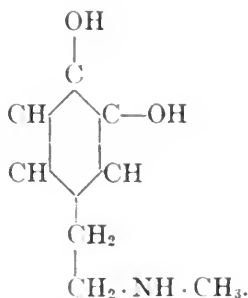
I turn now to another phase of the subject:

#### OTHER DRUGS POSSESSING A DILATING ACTION

A considerable number of bodies have been shown by different observers to possess a sympatho-mimetic action. It has appeared to me to be worth while to try out a few of these.

3 : 4 *Dihydroxyphenylethylmethylamine* ("Epinephrine"): This substance which I have obtained from Burroughs, Wellcome and Company, has the following formula:





It closely resembles epinephrine both in chemical structure and in pharmacological properties, being, however, only about one-tenth as active as the natural laevorotatory epinephrine. It has also seemed to me to have less stimulating action upon the heart. The rise in blood pressure produced by "epinine" seems, however, to be rather more prolonged than that which follows epinephrine. On the bronchi I find that its action in comparable doses is quite similar to that of epinephrine. The dilatation comes on promptly but is probably not quite so extensive as that produced by the natural active substance.

*Synthetic suprarenin.* This substance which was first prepared by Stolz and which I have obtained from the Farbwerke-Hoechst Company (New York), is claimed to be identical with the natural laevorotatory epinephrine. A number of authors have found its action to be fully equal and perhaps superior to that of the ordinary natural preparations on the market. I have also found this to be true in my own experience. On the bronchioles this synthetic suprarenin produced a prompt and extensive dilatation. The dilatation disappeared in practically the same time as that caused by the natural active substance.

*Cholin chloride.* In 1911 an extensive research was carried out by Hunt and Taveau<sup>26</sup> upon the vascular action of choline and a considerable number of its derivatives and related compounds. Previous observers have obtained very variable results with choline chloride, some observing a fall in blood pressure, while others obtained a marked rise, especially if atropine had been previously

<sup>26</sup> Hunt, R. and Taveau, R. de M., Bull. No. 73, Hyg. Lab., U. S. Pub., Health and Marine Hosp. Serv., Washington.

administered. I do not care to enter into a discussion of these previous observations further than to say that a comparison of my own findings with those described by a number of authors has led me to believe that several of these investigators must have worked with impure or deteriorated preparations, and further that the anaesthetic used (ether, chloroform, choral, paraldehyde, etc., and particularly *curare*) in all probability may exercise some influence upon the action of the substance. In my own work I have used fresh cholin chloride imported from Kahlbaum. With small doses I have obtained only slight effects, but with larger quantities I have observed a marked rise in blood pressure and a very extensive dilatation of the bronchioles (fig. 5). I should emphasize that these results have been obtained in animals (dogs) in which the brain and medulla had been destroyed by chloroform, and the vagi nerves were sectioned as a special precaution against any central influence on the bronchi. The spinal animals were thus entirely free from any confusing influence which might arise from the action of anaesthetics or *curare* upon the various organs and structures of the body. It appeared that under these conditions the action of the cholin chloride in fair sized doses was quite similar to that of epinephrine. But it seemed that the broncho-dilatation (after pilocarpine constriction) was probably slightly more extensive than that produced by epinephrine. It is very difficult, however, to positively verify this finding.

It has been noted by several observers that choline chloride is much more active in causing a rise of blood pressure after atropine has been administered to the animal. In most of my experiments I have found it necessary to give small injections of pilocarpine to the animals just before the choline chloride was injected in order that a broncho-constriction might be produced. I do not believe, however, that the pilocarpine exercised any special influence upon the action of the choline chloride. The dilatation of the bronchioles by choline makes it appear almost certain that the drug acts by stimulating the endings of the broncho-dilator nerves, for a marked rise in blood pressure occurs at the same time, and in the absence of any medullary action, this rise seems to be almost certainly due to a peripheral vaso-constriction probably pro-

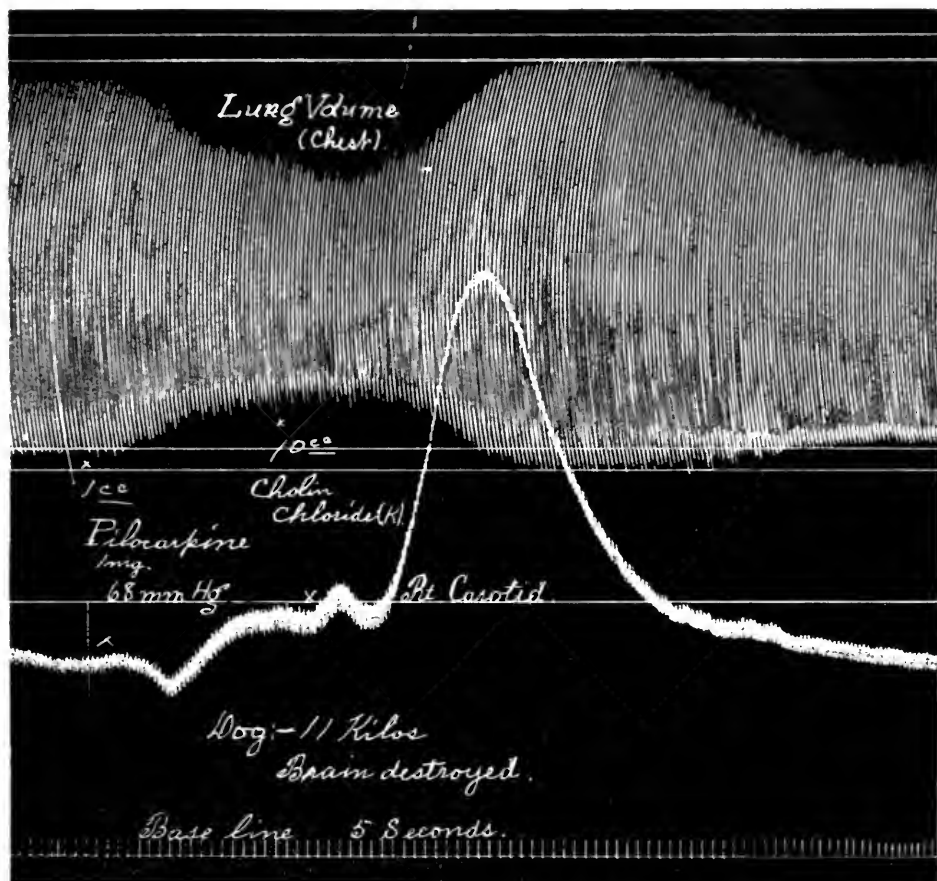


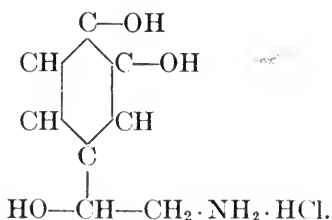
FIG. 5. LUNG VOLUME AND BLOOD PRESSURE. SPINAL DOG

At the left of the tracing pilocarpine was injected and produced a broncho-constriction. Later choline chloride (Kahlbaum) was injected and caused a rise in blood pressure and a marked dilatation of the bronchioles.

duced in the same manner as that following injections of epinephrine. As above stated, however, I have not had an opportunity to verify these conclusions on animals with the central nervous system intact.

*Trimethylamine hydrochloride.* With this body I have also observed very marked dilatation of the bronchioles when these had previously been in a state of contraction (fig. 6). This substance is quite poisonous and very small doses show the dilating action well. At the same time a marked rise in blood pressure occurs. The general resemblance of these results to those obtained with epinephrine make it appear evident at once that these two substances probably act in a similar manner. The preparation of trimethylamine hydrochloride used in these experiments was fresh and was imported from Kahlbaum.

*Other dilating substances.* In consideration of the fact that at least four different substances, namely, epinephrine, "epinine," choline chloride and trimethylamine have been shown to cause an *active dilatation* of the tonically constricted bronchioles, it seems very probable that a number of other substances may act in a similar manner. In general we should expect these bodies to belong to the group of substances possessing a "sympathomimetic" action. A considerable number of such bodies have already been examined. Among these there are at least two which I feel we might fairly safely expect to cause an active dilatation. These are the orthodioxypyphenylethanolamine<sup>27</sup> whose chloride is known commercially as "arterenol" and whose formula may be represented thus:



<sup>27</sup> Cf. Schultz, W. H., Bull. No. 55, Hyg. Lab., U. S. Pub. Health and Marine Hosp. Serv., Washington.

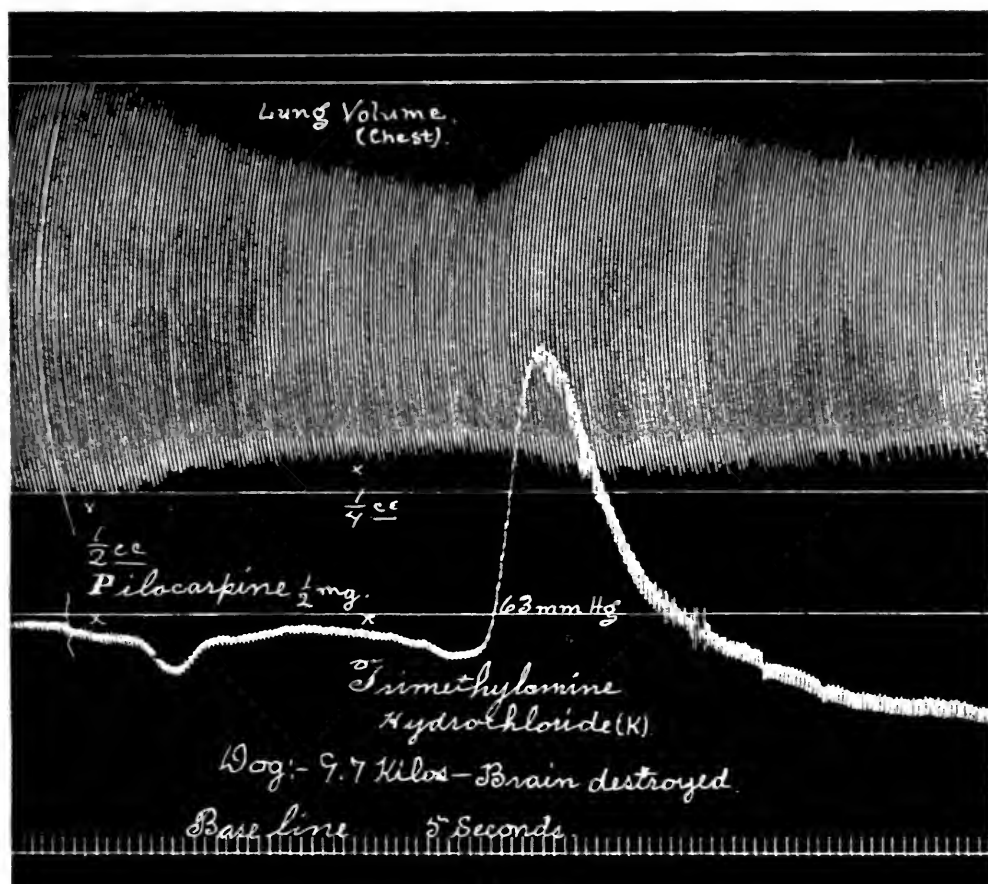
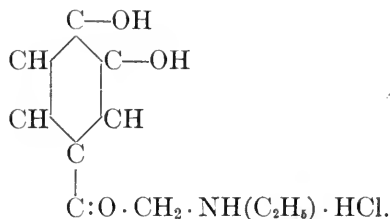


FIG. 6. LUNG VOLUME AND BLOOD PRESSURE. SPINAL DOG

At the beginning of the tracing pilocarpine was injected and caused a broncho-constriction. Trimethylamine hydrochloride (Kahlbaum) was then injected and produced a rise in pressure and a dilatation of the bronchioles.

and ethylaminoacetocatechol whose chloride is known commercially as "homorenon." Its formula may be represented thus:



Of these two bodies the first possesses a blood pressure raising activity fully equal to that of natural laevo-epinephrine, while "homorenon" is only one-eightieth as active (Schultz). Aside from the *actively dilating* substances which we may believe act in general by stimulating the broncho-dilator nerve endings, there are probably a considerable number of substances possessing an atropine-like action and which may produce a *passive dilatation* by paralysis of the broncho-constrictor nerve endings. With these latter substances I do not care to deal in this paper.

*The relative extent of dilatation under artificial and natural respiration.* It is rather difficult to determine this point and I have not so far been able to devise an experiment which would fully clear up the question. But I believe that clinically relief from spasmodic bronchial asthma has been obtained by the hypodermic injection of epinephrine under conditions in which probably not enough of the epinephrine could have reached the bronchioles to show a dilatation by ordinary experimental methods with (positive) artificial respiration. In ordinary experiments it is difficult to produce a dilatation by subcutaneous or even intramuscular injections of epinephrine. In natural respiration I suspect that the dilatation occurring from a given dose of epinephrine or choline chloride, etc., is much greater than would result under (positive) artificial respiration. There are a number of reasons for this. Under natural conditions the aspirating action of the respiratory movements upon the lungs is probably of much help by tending to overcome the natural elasticity of the lung tissues, to

increase the blood flow into the lungs, etc. And this dilating force is naturally distributed over the whole lungs. When air is forced into the trachea then the pressure of this air must overcome a series of influences all of which are tending to produce a bronchial constriction. Aside from whatever bearing these mechanical influences may have, it seems probable that the vital reactions of the bronchioles in a normal animal are quite different from those of one reduced to ordinary experimental conditions. I may briefly discuss this under the heading of

*The delicacy of bronchial responses.* During the preparation of this paper I have been much pleased by the appearance of an article by Douglas and Haldane<sup>23</sup> in which they show by a comparison of (1) the average composition of the expired air, (2) that of the alveolar air, and (3) the average volume of each breath, that during the hypernoea of muscular work there is a very great increase in the volume of the effective dead space in breathing; and they conclude that this increase is due to dilatation of the bronchi with a consequent diminished resistance to the passage of air inwards and outwards.

It is a matter of common experience in every pharmacological laboratory that the intravenous injection of almost any drug will cause some immediate change in the respiration of the animal. If the dose be small the change of course may be slight, but the more sensitive the method of recording and the lighter the anaesthesia, the surer will be the appearance of the alteration. Temperature changes are probably often concerned. It is, however, easy to observe that with a (probably large) majority of drugs the respiration is more sensitive than the circulation. And my experience with this series of spinal animals has indicated quite clearly to me that if our methods of experimental procedure are made sufficiently sensitive, we may almost certainly expect to detect in the bronchioles a great many drug responses which are wholly of peripheral origin. I should perhaps mention in this connection the peculiar more or less rhythmic variations in bronchial

<sup>23</sup> Douglas, C. G. and Haldane, J. S., *Journal of Physiology*, 1912, xlv, no. 4, pp. 235 to 238.

tone which often produce a series of undulating waves in the lung volume tracings. These are often accentuated by pilocarpine,<sup>29</sup> while epinephrine tends to depress them. And they are probably almost as marked in the spinal animal (with both vagi sectioned) as in the (etherized) animal whose brain and vagi are intact. When in addition to these reactions in the unconscious animal we consider what influence the varying play of central impulses in the conscious animal may have on the bronchioles, the significance of the results of Douglas and Haldane at once become evident, and it is extremely probable that in the normal state a great variety of reflex activities may coöperate to help maintain the tone of the bronchial musculature in a condition of almost constant variation.

#### CONCLUSIONS<sup>30</sup>

1. A new method for the study of volume changes in one lung is described. This consists in placing a specially designed metal plate between the lung and the pericardium in such a manner that the whole lung may be left freely movable in a cavity formed by the metal plate on the mesial and diaphragmatic sides and by the chest wall on the outer side. This cavity serves as a plethysmograph for the lung.

2. A method for maintaining the blood pressure of an animal at a constant level after its blood has been rendered incoagulable by hirudin is described. This procedure makes it possible to study the action of various substances upon the lungs in situ when the confusing influence of enormous changes in blood pressure have been eliminated. This method may also be used under certain conditions for the study of volume changes in such organs as the kidney, spleen, intestinal loop, hind limb, etc., when the organ, while maintaining its natural relations, is enclosed in an oncometer. For the study of reactions in the organs themselves

<sup>29</sup> Prevost, J. L., et Saloz, J., *Archives Internationales de Physiologie*, 1909, viii, p. 333.

<sup>30</sup> Just as this paper is ready for the press an excellent article on the bronchodilator nerves in the cat has been published by W. E. Dixon and Fred Ransom, *Journal of Physiology*, 1912, xiv, p. 413.



the entering nerves may be sectioned or painted with cocaine or phenol.

3. Under these conditions epinephrine produces a prompt dilatation of the bronchioles if these had been in a state of contraction when the drug was injected. Hence the broncho-dilator action of epinephrine is wholly independent of the marked rise in blood pressure which occurs when the drug is injected under ordinary conditions.

4. Moderate quantities of atropine do not paralyze the broncho-dilator nerve endings, but large doses (particularly if vanadium has been administered) seem to depress the endings to an appreciable extent.

5. The injection of a small dose of pilocarpine into a *fresh* spinal dog whose vago-sympathetic nerves have been sectioned low down in the neck generally produces a slight initial broncho-constriction which is soon followed by a moderate but usually prolonged dilatation. Under corresponding conditions the secondary dilatation does not occur if the suprarenal glands have been excised or clamped off before the pilocarpine is injected. This paradoxical dilatation which is also apparently produced by muscarine, seems to be identical in origin with a number of other paradoxical reactions occurring elsewhere in the body when the suprarenal glands are intact and may be stimulated to an increased secretion of epinephrine by the pilocarpine. Larger doses of pilocarpine cause constriction of the bronchioles in all animals.

6. Several alkaloids including quinine (and even atropine itself after the broncho-constrictor nerve endings have been paralyzed) cause broncho-constriction. This appears to be a direct muscular action.

7. After a brief initial constriction nicotine causes a dilatation which appears to be due to an increased secretion of epinephrine caused by the nicotine. But if after complete ganglionic paralysis by nicotine a constriction be produced by pilocarpine, then the injection of more nicotine causes a slow, prolonged, but very complete dilatation. The action of the nicotine under these conditions is unexplained.

8. Sodium iodide and camphoric acid do not affect the bronchial nerves. Agaricin seems to depress the broncho-constrictor endings.

9. The 3 : 4—dihydroxyphenylethylmethylaniline ("epinine"), synthetic adrenalin (Farbwerke-Hoechst Company), choline chloride and trimethylamine hydrochloride all produce active broncho-dilatation. The orthodioxypheylethanolamine hydrochloride should also probably be included here.

10. It is very probable that in the normal state and with natural respiration the dilator response of the bronchioles to small quantities of epinephrine is very much more effective than is generally found to be the case in experimental animals, for evidently relief has been obtained clinically from spasmodic bronchial asthma by the hypodermic injection of epinephrine under conditions in which it is extremely probable that not enough of the substance could have reached the bronchioles to produce a dilatation under ordinary experimental conditions with artificial respiration.

FURTHER OBSERVATIONS ON FUNGI, PARTICULARLY  
*CLITOCYBE SUDORIFICA* PECK, *PHOLIOTA AUTUM-*  
*NALIS* PECK, AND *INOCYBE DECIPIENS* BRESADOLA

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In a previous communication (1) it has been shown that the fungus described by Peck (2) as the variety *sudorifica* of the species *Clitocybe dealbata* Sowerby, from plants collected by Mr. F. G. Howland, of Saratoga Springs, New York, contains a poison with a muscarine-like action upon animals. An extract of the fungus kills rabbits and guinea pigs in a few hours with salivation, diarrhoea, discharge of urine and convulsive movements, and when dropped upon the conjunctiva of the rabbit's eye causes a constriction of the pupil, relieved by atropine. This muscarine-like action is brought out particularly well upon the frog's heart which is stopped in a few seconds in diastole, the heart's action being completely restored by atropine. The ingestion of this fungus by man, it will be remembered, produced a profuse perspiration lasting about five hours without however other ill effects. In a later communication Peck (3) has called attention to the poisonous properties of this plant, raising it to specific rank under the name *Clitocybe sudorifica*.

We have recently received a further supply of this *clitocybe* from Dr. Peck and have begun chemical investigations in the hopes of isolating muscarine from it. For this purpose we have followed the Harmsen method as modified by Clark and Kantor (4). In this method the fungi are twice extracted with 95 per cent alcohol, the combined solutions being evaporated to a small bulk on a water bath and again extracted with alcohol. The second extract is now evaporated to the consistency of a thin syrup which is triturated with powdered glass with the resultant formation of

a stiff paste. This paste is dried over sulphuric acid in a vacuum desiccator and after a week's time extracted with three successive portions of absolute alcohol. These solutions being combined, they are evaporated to dryness on a water bath, treated with small quantities of water which gives a clear solution and leaves behind the fatty matter in the extract. This solution contains practically all the muscarine present in the plant and by using this method Harmsen has succeeded in extracting it quantitatively from *Amanita muscaria*.

By applying the same method to *Clitocybe sudorifica* we obtained a final product in which small portions dissolved in water exhibited the characteristic muscarine action upon the frog's heart neutralized by atropine. The fungus residue left after the alcoholic extraction was quite without activity. The poison in *Clitocybe sudorifica* is therefore like muscarine capable of extracting from the plant by the Harmsen method. Lack of material has prevented the completion of this work by the application of specific precipitants for the purification of muscarine, but our original supposition that the substance present in this fungus with the action we have described is the muscarine of Schmiedeberg is considerably strengthened by this attempt at isolation. It may be further pointed out that the boiled fungus extract has the same action on the frog's heart as the raw, which holds also for *Amanita muscaria*. The similarity in the effect of *Amanita muscaria* and *Clitocybe sudorifica* upon the frog's heart is shown in charts I and II.

#### *Pholiota autumnalis* Peck

In a recent publication by Peck (5) attention has been called to the fact that *Pholiota autumnalis*, a mushroom not heretofore supposed to possess deleterious properties, was the cause of severe poisoning in a family in Minnesota consisting of three individuals, a mother and two children. The children both died, but the mother recovered. Through the kindness of Dr. Peck we received two lots of this fungus for study, one lot collected in Minnesota, and the other in New York. The Minnesota specimens proved entirely without action on rabbits and guinea pigs

and on the frog's heart. They did contain an agglutinin for rabbits' corpuscles, however, operative in a one to twenty dilution of an extract made from 1 gram of fungus to 10 cc. of normal saline. The New York specimens, while entirely devoid of action upon blood corpuscles, were acutely poisonous to both rabbits and guinea pigs. The strength of this poison is shown in the following table:

TABLE 1

*Toxicity of Pholiota autumnalis* Peck for rabbits and guinea pigs. Extracts made up in the proportion of 1 gram of dried fungus to 10 cc. of normal saline. Heated to 65°C. half an hour.

RABBITS				GUINEA PIGS			
No.	Weight	Dosage	Result	No.	Weight	Dosage	Result
		cc.				cc.	
I	1950	5	Death in 3 days	I	570	4	Death in 2 days
II	1720	4	Death in 1 day	II	350	3	Death in 1 day
III	1250	3	Death in 2 days	III	300	2	Death in 1 day
IV	1220	3	Death in 2 days	IV	210	1.8	Death in 1 day
V	1100	2	Death in 2 days	V	325	1.0	Death in 3 days
VI	1095	1	Death in 2 days	VI	320	1.0	Death in 4 days
VII	1260	0.5	Death in 1 day	VII	300	0.5	Death in 7 days
VIII	985	0.2	No effect	IX	200	0.5	Death in 3 days
				X	195	0.125	Death in 4 days
				XI	190	0.05	No result

At autopsy few changes of importance can be made out in these animals. There is usually a little oedema at the site of inoculation and punctiform hemorrhages appear in the abdominal muscles adjacent to this. Similar hemorrhages are found on the pleural surfaces of the lung and rarely in the cortical areas of the cerebrum. More extensive hemorrhages may occur in the lungs, leaving a mass of extravasated blood in the pleural sac, and similar collections of blood may be found in the peritoneal cavity. The heart is usually in diastole. The changes found resemble somewhat those seen in animals poisoned with *Amanita phalloides* and extracts of the pholiota are quite as poisonous as are extracts of the deadly amanita. The poison can also be obtained from the fungus by 65 per cent alcohol, an extract of the plant in alcohol of this strength evaporated to a small

bulk and taken up in water exhibiting the characteristic toxicity for guinea pigs and rabbits, killing them within forty-eight hours. In this property the poison resembles the *Amanita-toxin* which is best obtained from *Amanita phalloides* by 65 per cent alcohol. The *Amanita-toxin* however is also soluble in 95 per cent alcohol while the poison in *Pholiota autumnalis* goes into this solvent only in the faintest traces, the residue left after treatment having apparently the same degree of toxicity as the original material. The two poisons thus differ somewhat in their solvent properties.

Boiled extracts of *Pholiota autumnalis* exhibit almost the same toxicity for both rabbits and guinea pigs as extracts heated to 65°C. half an hour, although the poisonous effects may be slightly delayed in appearance. The plant has a peculiar action on the frog's heart both when applied locally and when injected subcutaneously. It produces little or no change in the rate but a gradual dilatation appears, this dilatation not being affected to any degree by atropine. As stated above the heart may be found dilated in rabbits or guinea pigs poisoned by subcutaneous inoculation.

From the evidence here adduced it is evident that *Pholiota autumnalis* Peck contains a powerful poison for both rabbits and guinea pigs. In view of this property and in view of the fatal accidents reported by Peck this species should be grouped with the deadly poisonous mushrooms.

#### *Inocybe decipiens* Bresadola

Previous investigations have shown that at least two species of *inocybe* are endowed with toxic properties. One of us (6) has described a peculiar anaesthetic or narcotic poison in *Inocybe infelix* and Clark and Kantor (l.c.) have found a somewhat similar poison in specimens of *Inocybe infida*, which could not be isolated from the fungus by the methods applicable to muscarine. We have recently received from Mr. Simon Davis of Brookline, Mass., small quantities of another species of *inocybe*, *Inocybe decipiens*, which also exhibits toxic action upon animals. Ex-

tracts of the plant made up in the proportion of 1 gram of material to 10 cc. of physiological salt solution contain an agglutinin for rabbits' corpuscles active in a dilution of one to ten. This agglutinin is destroyed by heating to 65°C. half an hour. The heated extract in quantities of 2 to 4 cc. kills guinea pigs acutely. At times the inoculation is followed almost immediately by the appearance of labored respirations and death may take place within twenty minutes. In such animals nothing can be made out at autopsy except a dilated heart. In other instances the animals develop within fifteen minutes to half an hour, excessive secretions from the eyes, mouth, and nose, together with the labored respirations. These symptoms last two to three hours and then gradually disappear, the animals usually succumbing within twenty-four hours, however, from the later effects of the poison. At autopsy little of importance can be detected except occasionally hemorrhages into the stomach. Similar effects are also produced in rabbits by inoculation with the fungus extract and local application of the extract to the conjunctiva is followed by a constriction of the pupil relieved by atropine. The action upon animals is almost identical with that shown by *Amanita muscaria*. This similarity to muscarine is brought out more strikingly by testing the fungus extract upon the isolated frog's heart. Both by local application and by subcutaneous injection it produces a stopping of the heart in diastole, this effect being completely neutralised by atropine both as to rate and rhythm. Boiling the extract does not rob it of this property. The close resemblance of *Inocybe decipiens* to *Amanita muscaria* is shown in charts I and III.

It is evident from these observations that *Inocybe decipiens* Bresadola contains a poison similar in its action to muscarine or at least belonging to the muscarine-pilocarpine series. As far as can be learned this fungus has not been tested for edibility. It should undoubtedly be regarded as a deadly poisonous mushroom.

*Agaricus amygdalinus* Curtis

This fungus, which is regarded by some authors (Farlow (7)) as identical with *Agaricus fabaceus* Berkeley, has occasionally caused unpleasant symptoms when ingested, but no cases of serious poisoning have been traced to it. Specimens were sent us for examination by Dr. Trelease, Director of the Missouri Botanical Station, St. Louis. Their extracts were quite without effect upon blood corpuscles, had no action upon the frog's heart and were not poisonous to either rabbits or guinea pigs on subcutaneous inoculation.

*Amanita pantherina* (De Candolle) Fries

Particular interest attaches to the examination of this fungus because of the divergence of opinion as to its properties in general and because of its rarity in America. Earlier authors, such as Stevenson (8) regarded it as a poisonous mushroom and Inoko (9) has obtained muscarine from the Japanese variety, which he believes represents *Amanita muscaria* in Japan. Melville and Macadam (10) state that writers are not in agreement as to its properties. Kobert (11) however, regards it as deadly poisonous and believes that it contains muscarine. Some question has arisen as to the occurrence of the true *Amanita pantherina* in America. The *Amanita cothurnata* of Atkinson resembles, according to this author (12) specimens of *Amanita pantherina* received from Bresadola of Austro-Hungary and may represent the American variety. Through the kindness of Mr. Simon Davis we received a few characteristic plants of this species gathered at Stow, Massachusetts. Their extracts were without effect upon blood corpuscles and exhibited no definite toxic action upon rabbits or guinea pigs. Tested upon the exposed frog's heart the extract produced some slowing of the rate but no stopping in diastole characteristic of muscarine, and the slowing which was produced was not neutralised by atropine.

It is, of course, unwise to come to any definite conclusion from the examination of a few specimens and it is quite possible that



other plants of this species collected in America might exhibit properties similar to those of the European or Japanese forms.

*Amanita mappa* (Batsch) Fries

A small quantity of this amanita was obtained through Mr. Simon Davis. It contained a small amount of an haemolysin, destroyed at 65°C. in half an hour. Local application was without action upon the frog's heart. Heated extracts had no effect upon rabbits but produced a chronic intoxication in guinea pigs from which the animals died in about ten days. There were no particular lesions at autopsy. The reactions of the plant suggested a close similarity to *Amanita phalloides* but lack of material made it impossible to work out this point more clearly. *Amanita mappa* is regarded as a poisonous mushroom by Stevenson (13), by McIlvaine and Macadam (14), and it is also so regarded by the majority of collectors. According to Kobert (15) it is probably a variety of *Amanita phalloides*, but he also states that it may contain muscarine. The specimens we have examined are certainly free from muscarine and closely resemble *Amanita phalloides* in their properties.

*Gyromytra esculenta* Fries

The European variety of *Gyromytra esculenta* or *Helvella esculenta* is regarded as a poisonous plant, the active principle of which, *Helvellic acid*, was isolated by Boehm and Külz in 1885 (16). Lövegren (17) has recently reported a severe poisoning in which a family of five were affected, a girl of five years dying four days after eating the fungi. The particular symptoms noted were vomiting, colicky pains, weakness, irregular respirations, tonic cramps in the voluntary muscles, dilatation of the pupils, jaundice and prolonged unconsciousness. At autopsy the chief lesions were a parenchymatous nephritis, fatty degeneration of the liver and excessive pigmentation of the spleen. It should be noted that the *Helvellic acid* of Boehm and Külz was powerfully haemolytic. The lesions described by Lövegren point to the action of a haemolytic toxin. No cases of poisoning from this

fungus have been reported in this country, as far as we know, and indeed considerable doubt exists as to whether the American plants of this species are poisonous. According to McIlvaine and Macadam (18) they have no bad effects when ingested.

A number of specimens of this fungus collected at Stow, Massachusetts, were sent me by Mr. Simon Davis. Their examination was entirely negative. Extracts of the fungus contained no haemolysin and no agglutinin, had no action upon the frog's heart and no poisonous effect upon guinea pigs or rabbits by subcutaneous inoculation.

*Entoloma modestum* Peck

No haemolysin. No agglutinin. No action upon frog's heart. Not poisonous to rabbits or guinea pigs by subcutaneous inoculation.

*Entoloma subtruncatum* Peck

No haemolysin. No agglutinin. Some slowing of the rate produced by local application to frog's heart, not neutralised by atropine. No poisonous action upon rabbits or guinea pigs by subcutaneous inoculation.

*Leptonia flavobrunnea* Peck

No action upon frog's heart, no toxic effect upon rabbits or guinea pigs by subcutaneous inoculation.

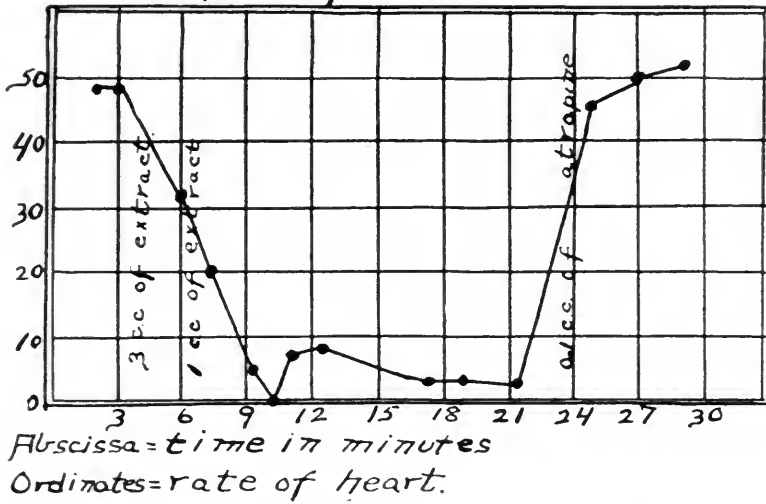
*Lepiota haemotosperma* Bulliard

No action upon frog's heart, no toxic effect upon rabbits or guinea pigs by subcutaneous inoculation.

We wish to acknowledge our gratitude to Dr. C. H. Peck of Albany, New York, Mr. Simon Davis, of Brookline, Massachusetts, and Dr. William Trelease, formerly Director of the Missouri Botanical Station at St. Louis, who have kindly supplied us with the material for this study.

## Chart I

Action of *Amanita muscaria*  
upon frog's heart



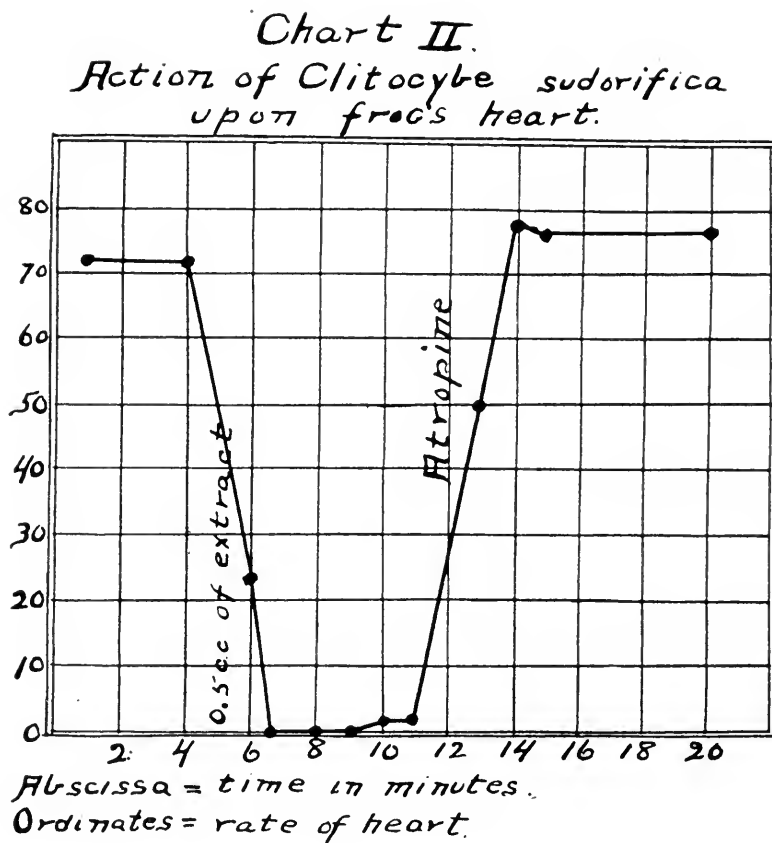
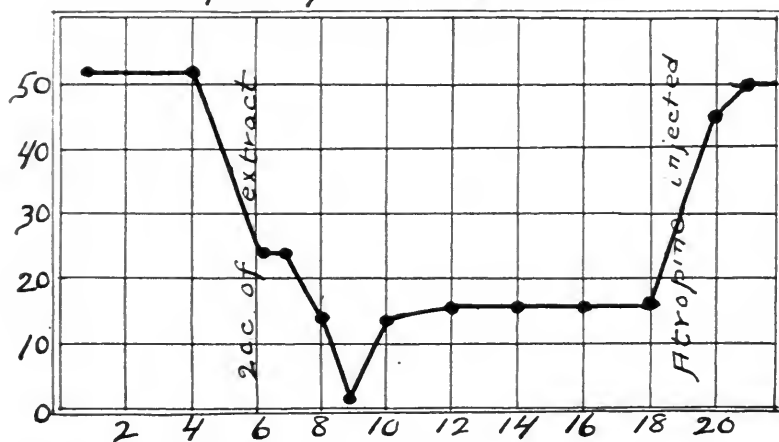


Chart III  
Action of *Inocybe decipiens*  
upon frog's heart.



Abscissa = time in minutes.

Ordinates = rate of heart.

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# ON THE RESISTANCE OF VARIOUS SPIROCHAETES IN CULTURES TO THE ACTION OF CHEMICAL AND PHYSICAL AGENTS

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The resistance of various microorganisms to the action of different chemical compounds and temperature had been thoroughly studied by many investigators, but that of spirochaetes remained so far undetermined, due to the lack of cultural methods. Recently one of us succeeded in isolating certain pathogenic and non-pathogenic varieties of the latter class of organisms in pure cultures and made it possible to find out how they would behave towards the action of some of the most commonly employed chemicals and therapeutic compounds. As our main interest is centered around the organism of syphilis we have chosen bichloride of mercury and Salvarsan as the principal substances for the present series of investigations.

The cultures used in these experiments were isolated by Noguchi, while the experiments of determination of the resistance of these organisms was made by Bronfenbrenner.

The cultures used for these experiments were grown on solid media as described by Noguchi and taken in a capillary pipette and broken up in a sterile flask with glass beads in the presence of 0.9 per cent sodium chloride solution, and filtered through a sterile filter paper free from agar.

One-tenth cubic centimeter of this filtrate is put in a small Petri-dish and 0.5 cc. of different chemicals in dilutions as indicated below is added so that the final dilutions are in fact by one-sixth higher than indicated in the tables. After a contact at room temperature for one hour the contents of the dishes are

TABLE I

	PALLIDUM (heavy type)		PALLIDUM (small type)		REFRINGENS		MUCOSUM		MICRODENTUM		BACTERIUM COLI	
	+	-	+	-	+	-	+	-	+	-	Inhibi- tion	Sterili- sation
HgCl <sub>2</sub> .....	300,000	200,000	700,000	500,000	300,000	200,000	400,000	300,000	400,000	300,000	16,000	16,000
As <sub>2</sub> O <sub>3</sub> .....	40,000	30,000	30,000	20,000	5,000	3,000	40,000	30,000	50,000	40,000	2,000	500
Trikresol.....	750	500	750	500	750	500	2,000	1,000	750	500		
Phenol.....	400	200	400	200	400	200	400	200	300	200		
Saponin.....	7,500	5,000	10,000	7,000	10,000	5,000	5,000	3,000	5,000	3,000	100	100
Sodium tauro- cholate.....	2,000	1,000	3,000	2,000	3,000	2,000	5,000	4,000	10,000	5,000	100	100
NaOH.....	$\frac{N}{40}$	$\frac{N}{30}$	$\frac{N}{30}$	$\frac{N}{30}$	$\frac{N}{30}$	$\frac{N}{20}$	$\frac{N}{50}$	$\frac{N}{30}$	$\frac{N}{20}$	$\frac{N}{10}$		
HCl.....	$\frac{N}{30}$	$\frac{N}{30}$	$\frac{N}{30}$	$\frac{N}{30}$	$\frac{N}{30}$	$\frac{N}{30}$	$\frac{N}{30}$	$\frac{N}{30}$	$\frac{N}{30}$	$\frac{N}{30}$		
Gentian violet	500	300	500	300	500	200	500	200	500	200		
Alcohol.....	> 10	> 10	> 10	> 10	> 10	> 10	> 10	> 10	> 10	> 10		
Old "606".....	2,000	1,000	1,500	1,000	1,500	1,000	2,000	1,000	3,000	2,000		



transferred with the sterile capillary pipette into tubes, and the constituents of culture media subsequently added.

On Table I the numbers in the columns under the plus sign indicate the dilutions of chemicals at which the organisms grew, and those under the minus sign indicate the highest dilutions which exerted sterilizing action.

The results of these experiments were taken according to a microscopical examination with the dark-field illumination after ten days incubation at 37°C.

In the case of *Bacterium coli* the concentrated chemicals were diluted by means of bouillon and after inoculation with two loops of uniform suspension of *Bacterium coli* incubated for twenty-four hours at the temperature of 37°C. The absence of turbidity in the culture tubes at the end of twenty-four hours was taken as a sign of inhibition of growth, and subsequent agar cultures were made to determine the actual sterilization. It is, therefore, under the heading of *Colon bacillus* on Table I that two columns are found, one of which indicates the dilution at which inhibition only occurs, and the other the complete sterilization as tested by subsequent agar cultivation.

The results tabulated above show the comparative sensitiveness of spirochaetes in general as being at least about twenty, and in a few instances even as much as one-hundred times greater than in the case of *Colon-bacillus* tested with the same chemicals.

The alcohol was not tested especially but as a control for gentian-violet which contained alcohol, and as it is shown even in as strong dilution as 1:10 alcohol does not sterilize spirochaetes.

As to the Salvarsan (or old "606") its sterilizing property is about equivalent to the same of the Neo-Salvarsan (see first column on Table III).

It was found that very minute quantities far below the sterilizing limits of antiseptics (phenol, trikresol, bi-chloride of mercury and arsenic compounds) exerted very marked stimulating action upon the growth of *Spirochaetes*.

In order to see whether the presence of protein in the ascitic fluid used for cultivation might have inhibited the sterilizing action of the chemicals the following experiment was performed:

A fixed amount of bi-chloride of mercury, representing several toxic doses and different amounts of ascitic fluid were added to the fixed amount of spirochaete suspension, and the volume was made uniform by means of a physiological solution. This mixture was allowed to stand at room temperature for one hour and then ascitic fluid, physiological salt solution and agar added in the quantities indicated on Table II. The tubes are then incubated at 37°C. and the results of the experiment are taken after ten days.

TABLE II

	CONTROLS						
	cc.	cc.	cc.	cc.	cc.	cc.	cc.
HgCl <sub>2</sub> $\frac{1}{20000}$ .....	0	0	0.5	0.5	0.5	0.5	0.5
Ascitic fluid.....	0	2	0	0.5	1	2	3
Salt solution.....	2.5	0	2.5	1.5	1	0	0
Microdentium.....	0.1	0.1	0.1	0.1	0.1	0.1	0.1

*One hour at room temperature*

Ascitic fluid.....	3	1	3	2.5	9	1	0
Salt solution.....	0	2	0	0.5	1	2	2

*Rabbit's tissue and 10 cc. of ascitic agar in each tube*

Results after ten days.....	+	+	-	-	-	-	-
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Thus it was found that the addition of ascitic fluid in the conditions of experiment does not interfere with the action of the chemical used.

In the experiments tabulated above we determined the sterilizing activity of Salvarsan by simply mixing it with the spirochaetes.

In order to see what effect upon spirochaetes Salvarsan may exert in the body we tried to change the plan of our experiments so as to approach its action in the living organism. For this purpose 0.1 of a gram of Neo-Salvarsan was dissolved in 10 cc. of distilled water (resulting in a dilution 1:100). Of this dilution 0.1 cc. was put into a series of tubes and 0.5, 0.7, 1 cc., 2 cc., etc., until 10 cc. of different diluents, as indicated on Table III, were added, resulting in dilutions of Salvarsan 1:500, 1:700, 1:1000, 1:2000, etc., until 1:10,000 respectively. The diluents used were:

Physiological salt solution, sterile liver extract, same extract boiled for fifteen minutes, and rabbit's defibrinated blood (whole). The tubes were then incubated for three hours at a temperature of 37°C.

At the end of incubation 0.5 cc. from each of the tubes was mixed in a Petri-dish with 0.1 cc. of cultures of spirochaetes on liquid media, and left at room temperature for one hour. After this the contents of the Petri-dishes were transferred, by means of sterile pipettes into tubes for cultivation, and 10 cc. of nutritive media and a piece of sterile rabbit tissue were added to each tube.

Two different strains of spirochaetes were used in this experiment, one a strain of pallidum belonging to heavy type, and the other a culture of spirochaeta refringens. The plus signs on the table indicate the dilutions in which at the end of ten days growth could be detected by means of a microscopic examination under the dark-field. The minus signs indicate absence of growth.

To see if the amount of Neo-Salvarsan in the tubes interfered with the growth a control tube was made by mixing, without previous incubation 0.1 cc. of the same spirochaete suspension with 0.5 cc. of Neo-Salvarsan in dilution 1:1000 in physiological salt solution, and as the table indicates, this amount of Neo-Salvarsan did not prevent the growth of spirochaete.

TABLE III

DILUTION OF NEOSALVARSAN IN DIFFERENT DILUENTS	PHYSIOLOGICAL SOLUTION		LIVER EXTRACT		BOILED LIVER EXTRACT		RABBIT'S BLOOD		CONTROLS	
	Refringens	Pallidum	Refringens	Pallidum	Refringens	Pallidum	Refringens	Pallidum	Refringens	Pallidum
1 : 700.....	-	-	-	-	-	-	-	-		
1 : 1000.....	-	-	-	-	+	+	-	-	+	+
1 : 2000.....	+	<+	-	-	++	++	-	-		
1 : 3000.....	++	++	+	<<+			-	-		
1 : 5000.....			+	+			<<+	-		
1 : 7000.....			++	++			+	<+		
1 : 10000.....							+	+		

The first column of the Table III is comparable to the corresponding one on the Table I where Salvarsan (old) was simply

diluted with physiological salt solution and mixed with spirochaetes. The columns 2, 3 and 4, however, give the idea of what may be taking place in the living organism. We see, especially from the column 4, that the presence of rabbit's blood increased the sterilizing capacity of Neo-Salvarsan about five times and the liver extract at least increased it twice. We see also that the same extract being boiled—not only loses its ability to increase the toxic effect produced by Neo-Salvarsan upon spirochaetes, but on the contrary lessens it. This fact suggests once more the validity of the hypothesis that Neo-Salvarsan is broken up in the living organism, and that its derivatives are especially toxic for spirochaetes. The substances in the body that are capable of splitting Neo-Salvarsan may possibly be of enzym nature because their activity is destroyed by boiling.

In order to study the effect of high temperatures upon spirochaetes the cultures were washed in physiological salt solution as above and 0.2 cc. of the resulting suspension, after filtration, was put into tubes and incubated in the water bath for different lengths of time at different temperatures as indicated on Table IV. After the incubation period in each case was completed a piece of sterile rabbit's tissue and ascitic agar were added to each tube and incubated at 37°C. for ten days, at the end of which period the contents of the tubes were examined by means of dark-field illumination. The plus signs on the table indicate the presence of growth, the minus signs indicate the absence of growth, or sterilizing action of the corresponding temperature.

TABLE IV

	ROOM TEMPERATURE				37° C.			40° C.		45° C.			
	0	2 h.	6 h.	12 h.	2 h.	3 h.	6 h.	30 m.	1 h.	3 m.	5 m.	7 m.	10 m.
Microdentium ....	+	+	<+	-	+	+	<+	+	+	+	<<+	-	-
Refringens.....	+	<+	<<+	+	+	+	-	+	+	+	<<+	-	-
Pallidum.....	+	+	+	<+	+	+	<+	+	+	+	+	<+	-
Pallidum heavy type.....	+	+	+	-	+	+	<+	+	+	+	<+	<<+	-
Mucosum small type.....	+	+	+	-	+	+	<+	+	+	+	<+	-	-

Although in a culture spirochaetes remain alive at room temperature for many weeks, yet as it results from above table at the end of twelve hours most of them are already dead. The reason for this is not the temperature, but the unfavorable conditions of experiment as: lack of nutritive substances, presence of oxygen, effect of light, the toxic effect of sodium chloride, etc., all of which markedly diminish the viability of spirochaetes. As the temperature is heightened the viability of spirochaetes suffers more, as we would naturally expect and even at the end of six hours at 37°C. we already see marked differences between the tubes exposed respectively to room and 37°C. temperature. At the temperature of 45°C. the rate of this process increases so much that already at the end of seven minutes most, and at the end of ten minutes all of the organisms tested were dead.

On the other hand, the spirochaetes survive for many hours at 45°C. if we subject them to this temperature under the favorable conditions such as are provided in the growing culture tubes, viz., strictly anaerobic conditions, properly balanced saline constituents and other nutrient substances.

#### SUMMARY

The toxic effect exerted by the chemicals in the experiments is from twenty to one-hundred times greater if tested upon spirochaetes than it is against *Colon-bacillus*.

The toxic effects of Salvarsan are increased from two to five times and possibly more in the presence of enzymes from the liver and especially from the blood.

Spirochaetes suspended in physiological salt solution are sterilized by the temperature of 45°C. in from seven to ten minutes.



SCIENTIFIC PROCEEDINGS OF THE AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

FOURTH ANNUAL MEETING

Medical Building of Western Reserve University, Cleveland, Ohio, December 30-31, 1912

*Edited by the Secretary, DR. JOHN AUER*

During the year 1912 the American Society for Pharmacology and Experimental Therapeutics lost by death two of its members, Dr. Waldemar Koch and Dr. Louis Nelson.

Dr. Waldemar Koch was Associate Professor of Pharmacology in the University of Chicago and a valued contributor to this Journal. His scientific researches are well known and need no mention in this place. He dared to attempt difficult tasks in new fields. By his untimely death the Society loses an earnest member and Science an effective worker.

Dr. Louis Nelson was Instructor in Pharmacology in the Harvard Medical School. The Society loses in him a young member full of promise.

To express its keen sense of loss in the death of these two members the Society passed appropriate resolutions and ordered them spread upon the record of its minutes.

*Further Observation on the Action of Caffein on the Circulation.*

William Salant. From the Pharmacological Laboratory. Bureau of Chemistry, United States Department of Agriculture.

The intravenous injection of caffein into dogs under chloretone anesthesia shows that a single dose of 3 to 12 mg. per kilogram, given to an animal with a normally acting heart, has a stimulating effect. In the presence of disturbed heart action the larger

doses may act as cardiac depressants. Even moderate amounts of caffein, when repeated, usually decreased the action of the heart. When given simultaneously with or immediately after the introduction of 10 to 20 per cent of alcohol, the depressing effect of this substance was corrected by caffein. Such action was, however, not uniform. In some cases the frequency of cardiac contractions was increased without changing the force. Experiments were also carried out on cats under chloretone anesthesia. The heart action, as in dogs, was recorded by the myocardiograph. The results showed in all cases cardiac depression or irregularity when 6 to 10 mg. per kilogram were injected intravenously. The cumulative effect was quite marked, and irregularity was noticed more frequently under these conditions. Smaller doses have in some animals increased the rate of heart action without affecting the force of the cardiac beat. Caffein, 30 to 40 mg. per kilogram, was also injected into the stomach, but had then no effect on the heart. Cardiac depression was observed when such doses were introduced into the intestines. Alcohol caused depression of the heart, decreasing the force and frequency of the beat. The simultaneous injection of alcohol and caffein, even when small doses of the latter were given, caused still further depression of the force of the heart action, but the frequency was moderately increased in a small proportion of animals.

*The Influence of Temperature on the Toxicity of Caffein.* William Salant. From the Pharmacological Laboratory, Bureau of Chemistry, United States Department of Agriculture.

The experiments were performed on frogs under temperatures varying between 45 to 102° F. The minimum lethal dose of caffein was found greatest at temperature of 70 to 80° F. and least at temperature of 98 to 102, the former being about two to three times as large as the latter. The symptoms observed after caffein varied with the temperatures of the environment. At 45° F. the muscular effect was especially marked, rigidity and tonic spasms being observed even when very small doses were injected. At a temperature of 96 to 100° the spasms were clonic, and no muscular rigidity was observed.



*The Effect of High Protein Diet on the Growth of Transplantable Tumors of the White Rat.* S. P. Beebe and Eleanor Van Alstyne. From the Cornell University Medical School.

*The Physiological Action of some Methylated Purines.* Lafayette B. Mendel and R. L. Kahn. From the Sheffield Laboratory of Physiological Chemistry, Yale University.

In view of the possible physiological and pharmacological interest attached to compounds of the purine group, especially the methylated derivatives, an experimental study of a number of these which have been synthesized by Prof. C. O. Johns of the Sheffield Laboratory has been begun. The present report deals solely with the possibility of diuretic properties, investigated almost exclusively on rabbits. For comparison, reference may be made to the well known diuretic methylated xanthine derivatives: theophylline (2, 6, dioxy-1, 3 dimethyl purine); theobromine (2, 6, dioxy-3, 7 dimethyl purine); paraxanthine (2, 6, dioxy-1, 7 dimethyl purine); caffeine (2, 6 dioxy-1, 3, 7 trimethyl purine).

The following new purine derivatives failed to induce any noteworthy diuresis in doses of about 0.25 gram, viz.: 2, 8 dioxy-1 methyl purine; 2, 8 dioxy-9 methyl purine; 2, 8 dioxy-1, 9 dimethyl purine; 2, 8 dioxy-6, 9 dimethyl purine; 2 oxy-9 methyl purine; 2 oxy-6, 9 dimethyl purine; 2 oxy-8, 9 dimethyl purine; 2 oxy-6, 8, 9 trimethyl purine. Other phenomena and derivatives are under investigation.

*The Action of Certain Drugs on the Electrocardiogram.* J. A. E. Eyster and W. J. Meek. From the Physiological Laboratory of the University of Wisconsin.

This work was done on the isolated hearts of cats and dogs perfused with Locke's solution by Langendorff's method. The electrocardiograms were obtained by leads from the right auricle and apex of left ventricle to the thread galvanometer. The contraction of the two ventricles was recorded by myocardiographs attached to base and apex. The results were as follows:

1. Decrease of the extent of ventricular contraction by absence of calcium from the perfusion fluid of the isolated heart does not

affect the R complex of the electrocardiogram to any noteworthy degree. The T wave is markedly affected. The absence of calcium affects first mainly the left ventricle.

2. Increase in the extent of ventricular contraction by epinephrin likewise does not, in a noteworthy degree affect the R wave in any case. The T wave may or may not be affected. Epinephrin acts mainly, or in small amounts, apparently exclusively on the right half of the isolated ventricle. Notwithstanding the great inequality in the stimulation of the two ventricles, the R complex is not, and the T wave may not be at all affected. This is opposed to the view of Einthoven that the R wave is formed mainly by contraction of the right ventricle, and the view of Selenin that the electrocardiogram is a composite of the action of the two ventricles and its form depends on which ventricle preponderates in its contraction. Some other factor than unequal contraction of the two ventricles is necessary to explain variations in direction and size of the T wave.

3. The action of epinephrin in increasing the rate of stimulus production occurs only in the supra-ventricular portions of the heart, the ventricular rate is unaffected when the ventricle is beating independent of the auricles.

*The Intestinal Absorption of Alcohol.* Paul J. Hanzlik. From the Pharmacological Laboratory of Western Reserve University.

the quantitative absorption of ethyl alcohol from the intestinal tract has been studied on a plan similar to that devised for phenol and sodium iodide previously published. It has been found that when approximately 10 per cent alcohol is introduced into the intestinal tract of cats and dogs in doses of 10 cc. per kilo, that there is an arrest of absorption at the end of half an hour after injection and this independently of the percentage absorbed, which varies from 33 to 85 per cent, leaving from 15 to 67 per cent of the alcohol unabsorbed. A comparatively slight and almost negligible absorption continues for one or two hours.

The incomplete absorption of alcohol is not due to re-excretion, since the intestinal canal excretes a much smaller quantity

(0 to 0.14 gram) than that which remains in the intestine when absorption is arrested. There must, therefore, be an actual inhibition of absorption. This does not seem to be due to a strictly local action, for preliminary treatment of an intestinal loop with alcohol does not inhibit the absorption of subsequent portions of alcohol from the same loop. On the other hand, the absorption from the intestine is diminished 5 to 20 per cent by intravenous injection of 10 per cent alcohol. The effect, therefore, seems to be systemic. It is not, however, due to changes in the systemic blood pressure.

In addition, the following facts were established: (1) the absorption of alcohol differs for different individuals of the same species but the average was practically identical in the different animals studied, i.e., cats and dogs; (2) the extent of the area does not affect the absorption; (3) absorption is about the same for all divisions of the small intestine, although slightly higher in the stomach and greatest in the colon; (4) the absorption was scarcely influenced by the concentration of the alcohol, although a 10 per cent solution of alcohol is absorbed somewhat better than 95 per cent alcohol, which in turn is absorbed slightly better than 5 per cent and 50 per cent solutions; (5) necrotic agents such as formaldehyde and concentrated phenol; calcium chloride, which diminishes the permeability of cells; and sodium fluoride, which acts as a protoplasmic poison, all diminish the absorption. Agents which cause visible anemia tend to lessen the absorption, but those which cause visible hyperemia tend to increase it but slightly; (6) post-mortem absorption from intestinal loops and stomach is comparatively good, averaging about 19.6 per cent.

The curious inhibition of absorption exhibited by alcohol was also observed with phenol and sodium iodide. With phenol the arrest of absorption was found to be due to an interference with the local circulation produced by the substance systemically or by local contact. In the case of the iodide, the interference in absorption appears at the end of 10 minutes after injection and was found to be due to the action of the haloid on the cells of the mucosa by local contact only. The data for alcohol, so far, indicate that the mechanism of its inhibition resembles that of phenol.

*The "Toxic Dose" of the Salicylates According to Clinical Statistics.* Paul J. Hanzlik. From the Pharmacological Laboratory of Western Reserve University.

This study was undertaken with the hope of clearing up some of the traditions concerning the therapeutic use of salicylates. The clinical records of the medical wards of Lakeside Hospital were made available through the courtesy of the medical staff and hospital officials. The routine use of salicylates at the hospital consists in repeating the dosage (10 to 20 grains) every hour or two till the onset of "toxic" symptoms, i.e., gastric or ear effects. This toxic dose was tabulated from the records of about 400 adult individuals (sixteen years or over).

The chief results of the investigation are as follows: The mean toxic doses of the various salicylates for males and females, respectively, are: 180 and 140 grains of the synthetic sodium salicylate; 200 and 135 grains of the "natural" sodium salicylate; 120 minims of the methyl salicylate; 165 and 120 grains of the acetylsalicylic acid or "aspirin;" 100 and 83 grains of the salicylosalicylic acid or "diplosal." It is seen that the salicylic esters are effective in much smaller doses than sodium salicylate. The "toxic" (and incidentally, the therapeutically effective) dose of salicylosalicylic acid is about 50 per cent, that of methyl salicylate about 60 per cent, of that of the sodium salicylate. The toxic dose of salicylates for females is approximately 80 per cent of that for males, corresponding very well with the average adult difference of weight in the two sexes (Vierordt's Tabellen).

The toxic dose of the synthetic salicylate for the majority (68 per cent) of male and female individuals lies between 100 and 200 grains. Practically the same holds true for the methyl salicylate. As to the other salicylates, the cases were too few to justify any conclusions except those shown by their general average. The toxic dose of the different salicylates is not influenced by ages between sixteen and seventy-five years. It is about the same for individuals of the black and white races. The toxic dose of the synthetic salicylate, and probably of the other salicylates, is the same in various diseased conditions of both sexes. The thera-

peutic response in various diseased conditions does not modify the toxic dose of the synthetic salicylate. The therapeutic efficiency of the synthetic salicylate is greatest in acute rheumatic fever. Albuminuria after toxic doses of the synthetic salicylate was much more frequent in febrile than in afebrile conditions suggesting that the renal irritation is due rather to the fever than to the salicylate. A certain number of male and female adults show idiosyncrasy, either abnormal tolerance or susceptibility, toward the ordinary toxic doses. No connection was found between these idiosyncrasies and the factors of age, sex, race and diseased condition.

*The Effect of Hematin on the Circulation and Respiration.* W. H. Brown and A. S. Loevenhart. From the Pharmacological Laboratory of the University of Wisconsin.

The hematin employed was made by the method of Schalfjew. The hematin was dissolved in a solution containing 0.85 per cent sodium chloride and 1.5 to 2 per cent sodium bicarbonate. The effects of hematin on the circulation and respiration, when injected intravenously in dogs and cats, may be briefly summarized as follows:

1. Small doses of hematin may cause a slight rise of blood pressure.

It seems probable that small doses of hematin stimulate the vaso-motor center.

2. Large doses of hematin cause a profound fall of blood pressure which is very prolonged. The principal factor in this fall of blood pressure is the marked dilatation of the splanchnic vessels. The splanchnic dilation either does not occur at all or but very slightly if the splanchnic nerves are cut. The splanchnic dilatation is partly compensated for by the marked constriction of cutaneous vessels and it seems probable that the cutaneous constriction is active and not simply passive to the splanchnic engorgement. The heart at first dilates and later shows increase in tone above the normal under hematin. The cardiac output for a time is greatly diminished.

3. Hematin in the large doses employed by us depresses the respiratory center and death under the drug is due to the paralysis of this center.

*The Effect of Anesthetics on the Output of Urine in Uranium Nephritis.* Wm. de B. MacNider. From the Laboratory of Pharmacology, University of North Carolina.

Following the injection of uranium nitrate subcutaneously the dog develops an albuminuria with casts and erythrocytes, and usually within twenty-four hours following the first injection the animals become glycosuric.

With the occurrence of the glycosuria, the amount of urine shows a very decided increase.

When such a nephritic, glycosuric and polyuric animal is anesthetized by Grehant's anesthetic or by morphine-ether, the output of urine following the anesthetic is influenced by both the anesthetic employed and by the age of the animal.

When Grehant's anesthetic is given in full strength, or in 60 per cent strength, to full grown dogs, the age of the animals, as near as could be ascertained, ranging between two and six years, the animals develop an anuria which is uninfluenced by diuretics.

If Grehant's anesthetic is employed in the same strength for young animals, puppies three and a half to five months old, which have been rendered nephritic by the same quantity of uranium per kilogram, the animals do not become anuric and are responsive to diuretics.

In the second series of animals morphine-ether was the anesthetic chosen.

When this anesthetic is employed for puppies and young dogs which have been rendered nephritic by uranium in the same quantity per kilogram as was used in the first series of animals the animals do not become anuric. The same statement holds true for the use of this anesthetic in full-grown animals, and in this class of animals the effect of morphine-ether shows a striking difference from the effect of Grehant's anesthetic. The animals are not rendered anuric and are responsive to diuretics.

In two experiments however, which were performed on very old animals nephritic from uranium, in which morphine-ether was used as the anesthetic, the animals became anuric and the anuria persisted uninfluenced by diuretics.

The kidneys of those animals which were rendered anuric by Grehant's anesthetic or by morphine-ether showed a very pronounced fatty degeneration of the cells of the straight tubules of the medulla and of the distal convoluted tubules and an acute swelling, non-fatty in nature, of the cortical tubules.

The kidneys of those animals, which were not rendered anuric by these anesthetics, showed fatty changes of much less severity and the acute swelling of the epithelium was slight or absent.

*The Physiological Assay of Aconite.* George B. Roth. From the Pharmacological Laboratory, University of Michigan.

Six physiological methods for the assay of aconite were investigated, three of which were found to be very unsatisfactory, namely, the toxic frog method, the one hour frog method and the effect upon the blood pressure of the cat or dog.

The two methods which gave the most satisfactory results were the toxic guinea pig method and the Squibb method. A general parallelism between these two methods was noted.

The production of increased tonus in the isolated frog's heart when it was perfused with aconitin in Ringer's solution was found to be more sensitive than any other test, the phenomenon having been obtained from as high a dilution as 1 in 2,000,000 of aconitin. It possessed the disadvantage, however, of not being useful for all galenicals.

*Renal Function in Experimental Passive Congestion.* L. G. Rowntree and R. Fitz.

*The Effect of Temporary Occlusion of Renal Circulation on Renal Function.* R. Fitz and L. G. Rowntree.

Both articles are now in press and will appear shortly in the Archives of Internal Medicine.

*Observations on Three Fungi not Previously Supposed to be Poisonous.* William W. Ford. From the Laboratory of Hygiene and Bacteriology, Johns Hopkins University

Three different species of mushrooms, not heretofore supposed to possess poisonous properties, have been studied during the past year and found to be highly toxic to animals. One of these, *Clitocybe sudorifica* Peck, has been eaten in small quantities by several individuals and was followed in all instances by profuse perspiration. This mushroom contains a poison acting upon animals like muscarin, and like it producing diastolic stopping of the heart which can be neutralized by atropin. A second fungus, *Pholiota autumnalis* Peck, has been the cause of several fatalities in children recently. It contains a heat resistant poison very active for rabbits and guinea pigs, resembling somewhat the Amanita-toxin in its action and like it capable of extraction from the plant by 65 per cent alcohol. The third fungus, *Inocybe decipiens* Bresadola, is not usually regarded as edible by mycologists and no cases of poisoning from its ingestion have been reported. It contains a muscarin-like poison however and should with the two others mentioned be classed among the deadly poisonous fungi.

*The Action of Bland Oils on Certain Hemolytic Agents.* J. D. Pilcher. From the Pharmacological Laboratory of Western Reserve University.

Alcohol, ether and chloral hydrate cause hemolysis. Corpuscles shaken for fifteen to thirty minutes with about 10 per cent of bland oils, such as olive, linseed, cottonseed and petrolatum liquidum, are more readily laked by these agents. In this respect olive oil is the most, and petrolatum liquidum the least active agent.

Saturating alcohol with these oils increases its hemolytic power as regards both plain and oiled corpuscles. This holds true for corpuscles treated with oils other than the oil dissolved in the alcohol.

Ether plus 10 per cent olive oil lakes plain corpuscles somewhat



more rapidly than does plain ether, but lyses oiled corpuscles somewhat less rapidly than plain ether. Equal parts of ether and olive oil lyses but slightly more rapidly than plain ether.

Saturation of the aqueous chloral solution with olive oil (which takes up very little of any oil) increases the hemolytic power of the former.

It is suggested that the oils increase the permeability of the lipoid (cholesterin, lecithin) cell envelope to the laking agent. A large excess of oil may decrease laking by attracting the laking agent, thus preventing its penetration into the cell.

*The Effect of Histamin upon Surviving Arteries.* Henry G. Barbour. From the Pharmacological Laboratory of the Yale Medical School.

Has appeared in the preceding number of this Journal.

*Nitrous Oxide Sleep Compared with Normal Sleep—Brain Cell Studies.* George W. Crile and J. B. Austin. From the Western Reserve Medical College.

Rabbits when kept awake for one hundred hours showed physical changes in the brain cells characteristic of those of overwork, surgical shock and of emotional exhaustion. Substituting nitrous oxide anesthesia for sleep and during the séance agitating the animal to such extent that, were it not for the anesthetic, it would be awake, we found the brain cells were protected almost wholly against such exhaustion; but animals that were kept awake and showed physical exhaustion and brain cell changes, then were allowed some sleep, say eight hours, and others were given nitrous oxide anesthesia an equal time with sufficient agitation to have kept them awake had they not been anesthetized, showed the following: the brain cells of those who had normal sleep showed marked recuperation excepting in those cells that were in the stage of marked exhaustion. These were not altered by the single séance of sleep. The brain cells that had been under nitrous oxide anesthesia to a very small extent showed recuperation.

*The Action of Diphtheria Toxin on the Vaso-motor Center.* W. T. Porter and J. H. Pratt. From the Laboratory of Comparative Physiology, Harvard Medical School.

Rabbits were given such amounts of diphtheria toxin intravenously as to cause death on the third day. Death usually occurred forty to forty-four hours after the injection. The observations were begun on the morning of the third day, a few hours before the fatal issue was expected or when the animal exhibited toxic symptoms. The depressor, central vagus, cardiac vagus, sciatic and peripheral end of the left splanchnic were stimulated with measured induction currents. There was a marked fall of blood pressure toward the end of life. Even when the blood pressure had fallen to a low point the vaso-motor center was not paralyzed.

*The Effect of Surplus Cow Serum upon Complement Fixation with Infectious Abortion.* Frank M. Surface. From the Kentucky Agricultural Experiment Station.

In using the fixation of complement for the diagnosis of infectious abortion in cattle, it was found that the sera of certain cows gave complete fixation when small amounts of serum were used (0.05 or 0.02 cc.), but that if larger quantities of serum (0.2 or 0.1 cc.) were added, no fixation occurred. In studying this phenomenon further, it was found that fixation could always be inhibited by adding an excess of cow serum. The point at which such inhibition occurs varies in different cows. In all but two of the cows tested, 0.5 cc. serum was more than enough to prevent fixation. With these two cows, it was necessary to add 0.7 cc.

It was further shown that a surplus of a non-reacting serum would also inhibit the fixation. Thus, the phenomenon is not due to a surplus of abortion amboceptors, and, consequently, cannot be explained as a Neisser-Wechsberg phenomenon. In spite of certain theoretical difficulties, this latter explanation has been offered to explain similar inhibiting effects.

A large number of experiments were carried out which cannot be mentioned here. The results have shown that there is a sub-

stance remaining in cow serum, after inactivation at 56°C. for thirty minutes, which is able to unite with sensitized abortion bacilli, and thus prevent their union with guinea-pig complement. This substance is destroyed by heating the serum at 65°C. for thirty minutes. A large portion of this substance can be absorbed from the serum, by treating with sensitized abortion bacteria. It can further be shown to be united with the bacteria after these have been washed to remove all serum.

This substance thus behaves as an anti-amboceptor. Whether it is of the nature of a thermostable, bacteriolytic complement or of a complementoid was not determined.

The relation of this phenomenon to precipitation and to conglutination was discussed briefly.

*Studies upon the Long-Continued Administration of Adrenalin and Nicotin.* I. Adler and C. L. Alsberg. From the Laboratory of Dr. Adler and the Bureau of Plant Industry, United States Department of Agriculture.

*The Hemolytic Power of Various Plants.* C. L. Alsberg. From the Bureau of Plant Industry, United States Department of Agriculture.

*Demonstration of a Carbonator. for Quantitative Carbon-dioxide Therapy.* Yandell Henderson. The Physiological Laboratory, Yale Medical School.

*Some Further Observations on the Relation of Vital Stains to the Tubercle.* Paul A. Lewis. From the Henry Phipps Institute of the University of Pennsylvania, Philadelphia.

In a previous paper it was reported that trypan red possessed in high degree the property of penetrating to the center of the experimental fibro-caseous tubercle produced in the rabbit by inoculation of tubercle bacilli of human type. Isamin blue became concentrated in the fibrous zone.

In extension of these observations there have been examined a series of benzidin dyes to determine their penetrating power

and vital staining properties generally. A considerable number of a series of naphthamine blues and blacks of various letters courteously supplied by Kalle and Company were found to be vital stains and to become concentrated in the tubercle. None were superior in penetrating power nor equal in differential affinities to the two previously reported on.

Congo red was found in appreciable concentration in the caseous areas of the tubercle at a time when all of the other tissues were colorless. This is the only dye so far encountered in which this relation prevails.

In conjunction with Mr. Robert B. Krauss—assistant in chemistry at the Phipps Institute—a beginning has been made in the study of modifications of trypan red with the purpose of determining its carrying powers for substances which may possess pharmacodynamic action. It has been determined that compounds of trypan red with iodine, thymol, eucalyptol, guaiacol and iodoform can be prepared, and that in the case of certain of the compounds with each of these substances, the vital staining properties and the ability to penetrate the tubercle remain as in trypan red.

In the course of this work it has been found very useful to compare the activities of the chemical compounds formed with that of trypan red as against the trypanosome infection in mice. Incidentally it has been our experience that iodine however introduced into the trypan red molecule lessens its anti-trypanosome activity.

In this series of experiments it was found impracticable to use on a large scale the fibrocaseous tubercle which takes several months to prepare. It has been found that the acute tuberculosis produced in the rabbit's lung by the inoculation of virulent bacilli of bovine type gives equivalent information in most points. The experiments have been so arranged that the study of the affinities of the substances used was coincident with a therapeutic test in the face of a certainly and rapidly fatal infection. Neither trypan red nor any modification so far studied has had an appreciable influence in prolonging the course of the infection. Some substances seem to inhibit tubercle formation, and the animals die somewhat earlier than the controls and show a more diffuse process in the lungs.

*Differences in the Toxic Effects of Ether and Chloroform, as Observed under Intratracheal Insufflation.* T. S. Githens and S. J. Meltzer. From the Department of Physiology and Pharmacology of the Rockefeller Institute.

In studying the toxic effects of chloroform and ether, when administered by the method of intratracheal insufflation, we observed various differences in the course of the intoxication brought on by excessive doses of these anesthetics. We shall discuss here, however, only the differences in the toxic action of these drugs upon the function of respiration and blood pressure. At the outset we have to point out, that when administering the anesthetics by the insufflation method one of their dangerous effects is here eliminated; it is the danger which is bound to result from a partial or complete paralysis of the respiratory function. Under the method of insufflation life remains safe even when the animal is completely curarized. Observations may be carried on, therefore, even after spontaneous respiration is completely abolished. On the other hand, intratracheal insufflation carried on with ordinary, permissible air pressure, does not cause apnea, that is, the individual continues to carry on its own spontaneous respirations, which on tracings, are easily distinguished from the infrequent partial interruptions of the continuous insufflation of air.

The observations which we wish to report here briefly, are as follows: When using ether, a certain dose may be administered which is amply sufficient to keep the animal completely anesthetized, while respiration and blood pressure may remain practically unimpaired for several hours. When this anesthetic dose is exceeded, the first striking effect is upon the spontaneous respiration which may be rapidly abolished. For instance, if complete anesthesia has been accomplished by a dose which we term "one-half ether" or "three-fourths ether" and now "full ether" is turned on, the respiration may stop completely within one to five minutes. At this stage blood pressure is not impaired. When, however, "full ether" is continued the blood pressure begins to come down. The descent is very gradual and slow. It is rarely less than one hour, in some cases it may be even several hours,

before the pressure reaches the dangerous stage. At that stage the blood pressure may not be above 20 or 25 mm. of mercury and pulse pressure also may be considerably reduced. However, even at this stage when the ether is turned off, the pressure may begin to rise at once: although some time has to pass before the respiration returns, and there is still another interval before reflexes and consciousness return. The duration of the returning ascent is shorter than the duration of the descent. It is, however, not absolutely necessary to shut off the ether completely; a reduction to "one-half ether" is, as a rule, soon followed by a return to a degree of blood pressure which is sufficient to obviate danger.

The ready abolition of the respiration by ether is a practically important phenomenon; it may serve as an indication that the etherization has entered the toxic zone. It is a danger signal, and since hours may pass before the real danger will be arrived at, it is a safe and very valuable signal.

It is different with chloroform. In doses which are undoubtedly in excess of the anesthetic dose, respiration and blood pressure go down practically together and this in a comparatively short time. When the administered dose is only slightly in excess of the reliably anesthetic dose, the impairment of respiration does not set in as early as under ether intoxication; but when this sets in, blood pressure begins to fall also, although the respiration may in some cases cease fifteen or twenty minutes before the fall of blood pressure reaches the danger point. Respiration perhaps resists chloroform intoxication slightly longer than that of ether, while blood pressure is affected much more rapidly and profoundly by chloroform than by ether. The zone separating safe anesthesia and danger is narrow, and there is no reliable danger sign.

*The Influence of Decerebration upon Morphin Tetanus in Frogs.*

Thomas S. Githens. From the Department of Physiology and Pharmacology of the Rockefeller Institute.

Morphin given in sufficient dose to normal frogs causes tetanus which does not come on, however, until several hours or even days after the injection.

I have found that decerebration hastens the onset of tetanus and also causes a marked reduction in the amount of morphin required to induce tetanus.

The smallest dose with which tetanus can be induced regularly in normal frogs, at room temperature, is  $\frac{1}{3}$  mg. per gram. (10 mg. for a 30 gram frog). This tetanus comes on in about twenty-four hours.

In decerebrated frogs, at room temperature, tetanus comes on after such a dose in from one-half to six hours, and may be induced with certainty after six to twenty-four hours by a dose of 0.1 mg. per gram (3 mg. for a 30 gram frog).

When frogs are kept cold, tetanus can be induced by much smaller doses, as we have stated in an earlier paper. Thus intact frogs kept in the cold show tetanus after doses of  $\frac{1}{30}$  mg. per gram (1 mg. for a 30 gram frog). Tetanus comes on after such a dose in from eighteen to twenty-four hours. Decerebrated frogs show tetanus after such a dose in from four to twelve hours, and it may be induced with certainty by doses of  $\frac{1}{300}$  mg. per gram ( $\frac{1}{10}$  mg. for a 30 gram frog) after an interval of one to three days.

Frogs with the entire brain including the medulla destroyed, do not respond as well as frogs with the entire brain except the medulla destroyed.

*On the Effect of Sodium Bicarbonate and Sodium Chloride upon the Convulsions Produced by Heroin and Strychnin.* Israel S. Kleiner. From the Department of Physiology and Pharmacology of the Rockefeller Institute.

Langer has recently reported that the convulsant action of heroin may be prevented if a hypertonic saline solution is given by mouth. For example, a subcutaneous injection of 0.07 gram of heroin hydrochloride per kilogram usually provokes clonic convulsions in a few minutes, but if 40 cc. of a saturated sodium bicarbonate solution are given simultaneously with the injection of the drug the convulsions do not occur. The same result is attained by the administration of 50 cc. of 10 per cent sodium chloride. Langer says that a small fraction of subcutaneously

injected heroin is excreted with the feces, although the major portion is eliminated by the kidneys, and he believes that the administration of the hypertonic solutions, in the experiments just mentioned, has resulted in an increased elimination through the mucosa of the stomach, because of the osmotic conditions. Langer states further that the administration of bicarbonate had no effect on strychnin poisoning. Here the objection may be raised that the dosage which he employed, 0.8 mg. per kilo, is too great; 0.5 mg. per kilo giving nearly 100 per cent positive toxic results.

We were interested in this work because some time ago we found that the subcutaneous injection of large quantities of *isotonic* saline, and even the oral administration of a large amount of water reduced the toxicity of a simultaneous subcutaneous injection of strychnin. We therefore repeated the work of Langer.

We will only give a brief summary of our results. As to the prevention of the convulsant action of heroin by the simultaneous administration of sodium bicarbonate, we could not confirm Langer's report. Of eight rabbits which received 0.07 gram of heroin hydrochloride per kilo and 40 cc. of the saturated sodium bicarbonate solution, only one failed to get convulsions, and of the seven which did get convulsions three died. While of twelve controls (with 0.07 gram heroin per kilo and nothing per os) three had no convulsions and one of the remaining nine died. Nor did the controls experience the toxic effects sooner than the experimental animals.

We are, however, able to confirm Langer's experiments with heroin insofar as they concern the prevention of the convulsions by hypertonic solutions of sodium chloride. When 50 cc. of 10 per cent NaCl were given per os and, shortly thereafter, 0.07 gram of heroin hydrochloride subcutaneously, no convulsions occurred in any of the eight experiments performed. We will not at this time discuss the effect upon the other symptoms produced by heroin. The same procedure, however, failed in strychnin poisoning. Using the dose of 0.5 mg. per kilo, the administration of 50 to 100 cc. of 10 per cent NaCl per os did not lessen the toxicity of this drug. In seven experiments only two



rabbits had no convulsions and four of the other five died. A control series of six rabbits gave the following results: one did not get convulsions, five got convulsions, one of which died.

To sum up: we can not confirm Langer's report that oral administration of saturated sodium bicarbonate solution lessens the intensity or prolongs the duration of the toxicity of heroin; we can, however, confirm his observations that 10 per cent NaCl does prevent heroin convulsions, while strychnin convulsions are not prevented by oral administration of *hypertonic* solutions even when a smaller dosage of strychnin is employed than Langer used.

*The Influence of Pituitrin upon the Depressor Action of the Vagus Nerve in the Cat.* J. Auer and S. J. Meltzer. From the Department of Physiology and Pharmacology of the Rockefeller Institute.

From the experiments of Oliver and Schäfer it is known that after an injection of epinephrin stimulation of the depressor nerve remains without any effect only during the acme of the rise. When the blood pressure begins to fall again, the effects of stimulation of the depressor nerve become manifest. We have shown recently that pituitrin reduces the action of the depressor nerve more effectively than adrenalin. Stimulation of the depressor nerve in rabbits after an intravenous injection of pituitrin may remain without any effect, not only during the acme of the rise but also when there is no rise, or during the fall which often precedes the rise, or after the rise of blood pressure comes down again to its original height. The reduction in the depressor action may persist as long as fourteen or sixteen minutes.

It is generally assumed, that the trunk of the cervical vagus nerve in cats contains depressor nerve fibers and stimulation of the central end of that nerve causes a fall of blood pressure. We studied, therefore, the action of pituitrin upon the blood pressure effects of this nerve in a number of cats. We shall mention briefly at first, a few general observations we made in this study.

It is stated by some writers that in cats any stimulus brings out only a fall of blood pressure. In our experience, in two cats

out of seven, stimulation of the central end of the vagus caused a rise; in one it was caused by any strength of stimulus, and in the other a weaker stimulus caused a rise and a stronger caused a fall. After long experimentation on these animals (cutting the splanchnics, etc.) every effective stimulus caused a fall.

It was stated by Bayliss and repeated by others that the vagus depression differs from that of the pure depressor of the rabbit, in that during vagus stimulation the pressure begins to go up again during stimulation. We observed in some cases of prolonged stimulation of the vagus a continued fall without any tendency to a rise during stimulation.

We observed a stimulation of the central end of the vagus bringing on a good fall of blood pressure in an experiment in which both splanchnic nerves were cut. In this instance the reflex vaso-dilatation must have taken place in other regions than that supplied by the splanchnic nerves. We must mention here that in all our experiments both vagi were cut, and any fall could not have been brought on by reflex slowing of the heart.

A sufficiently large dose of pituitrin (Parke, Davis and Company) brought on in practically every case, first a comparatively abrupt short fall, then a gradual prolonged rise of blood pressure. Only in one instance, after an injection of only one cubic centimeter of the warmed pituitrin, no fall preceded the rise, which was very moderate. Both kinds of effects, fall and rise, seemed to increase with the increase of the dose. In the effect of the first injection, the rise was always greater than the preceding fall. With repetition of the injections, gradually the fall increased and the rise decreased.

We come now to the effect of the injection of pituitrin upon the depressing action of stimulation of the central end of the vagus. We shall be very brief. Only when the rise produced by the pituitrin was very high and only during the acme of that rise had a stimulation of the vagus no effect. When the rise was not very high or during the ascent and descent of the rise, the depressing effect of a vagus stimulation was invariably definitely in evidence. Furthermore, under all circumstances the strongly reducing action of pituitrin upon the depressing effect of the stimu-

lation of the afferent fibers of the vagus disappeared in a much shorter time than in the action of pituitrin upon the stimulation of the depressor nerve in the rabbit. In some instances the effect was nearly all over in less than three minutes after the injection of pituitrin. It may be stated in general that the injection of pituitrin affects the depressor action of the vagus nerve in cats definitely less strongly than it does the action of the depressor nerve in rabbits. We shall not attempt to discuss here any theoretical explanation of the differences in the result.

*The Influence of Heat upon the Toxicity for Trypanosomes of Blood Containing Transformed Atoxyl.* B. T. Terry. From the Laboratories of the Rockefeller Institute.

The toxic substance (transformed atoxyl) formed when a solution of atoxyl in blood is incubated for two to three hours at 37°C., disappears to a large extent from the fluid in which the red blood corpuscles are suspended if, after dilution to prevent coagulation, this fluid while still containing the red blood corpuscles, is heated to 70°C. for ten minutes. Heating the same sample, or one not previously heated, to 100°C. for ten minutes, leaves the fluid almost as toxic for trypanosomes as if it had not been subjected to any subsequent heating at all, and very much more toxic than after heating to 70°C. for ten minutes. The evidence at hand indicates that heating to 70°C. for a short time causes the toxic substance to be taken up by the red blood corpuscles, while heating to 100°C. liberates it more or less completely. The liberation of the toxic substance is more complete after half an hour at 100°C. than after ten minutes.

*Variations in the Toxic Effect of Transformed Atoxyl on Trypanosomes in Vitro Caused by Altering the Number of the Organisms.* B. T. Terry. From the Laboratories of the Rockefeller Institute.

If atoxyl is transformed into a substance toxic for trypanosomes by first incubating it with blood for two to three hours at 37°C., and if the amount of the toxic substance formed is esti-

mated by determining in what dilution it will immobilize all of the trypanosomes in a given time, the influence of the toxic substance upon the parasites will be found to vary inversely with the number of organisms upon which it acts. When the number of trypanosomes is ten times as great in one suspension as in the other, dilutions of the toxic substance can be found which seem to have no influence on the suspension containing many parasites, while it exerts a markedly toxic action on the suspension containing the smaller number of organisms. From this it follows, (1) that the trypanosomes in suspension should be comparatively few if slight degrees of toxicity are to be detected, and (2) that a standard number of trypanosomes should be employed if great accuracy in comparing results obtained with different suspensions, is desired.

## ON THE PHARMACOLOGY OF THE RESPIRATORY CENTRE

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The action of the drugs on the respiration has a considerable importance from the aspect of practical therapeutics and is the subject of a large number of investigations. It is of no less interest from the purely biological aspect, for we have here a nervous centre which is constantly subjected to two different kinds of influences, the one, afferent impulses carried by the nerves, especially the fibers of the pneumogastric, the other of the nature of a chemical hormone, in carbonic acid and to a less extent oxygen-lack. The influence of drugs upon the reaction of the respiratory centre to carbonic acid has been investigated by some workers to whose results I shall return later, while the respiratory reflexes under drugs have comparatively seldom been examined, though it is generally stated that the members of the morphine series reduce the reflex excitability, and their use to allay cough is thus explained. While there can be no question of the relief given in cough by the use of morphine and its allies, the statement that this is due to depression of the reflex excitability is not supported by experimental observations and requires further investigation.

Various methods have been used to record graphically or measure the respiratory movements, but objections may be brought against most of them. Some workers have recorded only the movements of the diaphragm by means of a lever inserted between it and the liver, or by means of a slip of diaphragm muscle, but this gives only an imperfect picture of the actual amount of air inspired. The same criticism holds for the various methods

of recording thoracic movements by means of tambours, etc. A more popular method is the record of the breathing by various attachments to the tracheal cannula, such as tambours, which merely register the rate of respiration; elaborate pharmacological investigations have been made on rabbits by measuring the amount of air expired into flasks filled with water, the inspired air being separated from the expired by valves of various forms. But at the commencement of my experiments on rabbits I was struck by the change in the form and rate of the respiratory movements which were induced by the least narrowing or obstruction of the tracheal cannula. For example, the attachment of a very easily moving Chauveau valve close to the trachea of a rabbit deepens the respiratory movements greatly, and I am convinced that in any experiment on a rabbit in which the respiration is recorded or measured by apparatus attached to the trachea, dyspnoea is present throughout.

I have recorded the respiration by an adaptation of the plethysmograph. For a rabbit of 1000 to 1500 grams weight an oblong wooden box was used, measuring 28 cm. in length by 10 cm. in width and 9 cm. high. For cats and larger rabbits another box was used measuring 32 cm. in length by 12 cm. in width and 12 cm. high. The bottom and top were removed and the edges were ground perfectly smooth on sand-paper. A sheet of glass (*G*, fig. 1) was cut to fit over the box and could be attached air-tight to the top by means of vaseline. The ends of the box were cut out in the form of an arch. A sheet of very thin rubber sheeting was then attached along the sides of the box and continued over the arches at the end, and the joining made air-tight by glue and varnish. Above the attachment of the rubber a glass-tube *B* passed into the box, of which a transverse section is given in figure 1. It was divided by the rubber into two sections of which the upper, bounded by the glass above, rubber below, and the wooden sides and ends of the box communicated with the exterior only through the tube *B*. The lower section was bounded above and on both sides by a fold of the rubber. The tube *B* led to a float-recorder described by Jerusalem and Starling (1). A T-tube was inserted between the plethysmograph

and the recorder and connected with a small compressible air bulb by which air was pumped into the apparatus as desired. The tube connecting the plethysmograph and recorder was of 15 mm. calibre and 30 cm. in length.

Rabbits were used chiefly in my experiments and were anaesthetised with ether, decerebrated through a trephine opening and tied on their backs in the ordinary way, except that the two hind legs were generally tied at the end of the rabbit board instead of to the two sides. The plethysmograph was then detached from the recorder and placed over the animal, extending from the lower pelvis to the upper edge of the thorax. The

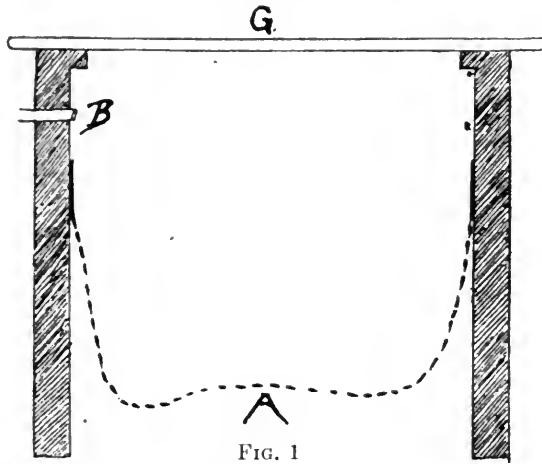


FIG. 1

glass top was removed and the rubber sheeting adjusted smoothly over the thorax and abdomen and the top was replaced and rendered tight with vaseline. At first some difficulty was met in including the whole thorax from the forelegs striking the apparatus, but this was overcome by cutting a small notch out of the front of the box and by attaching screws by which it could be raised from the rabbit board.

The trunk of the animal was thus completely enclosed, below by the rabbit board and to the sides and above by the rubber sheeting. As it breathed the sheeting moved up and down, and each inspiration drove the same amount of air out of the upper compartment of the box as was drawn into the lungs. The air

could escape only into the recorder and the excursion of the lever thus registers the actual amount of air moved at each respiration. There was no interference with the respiration from the recording apparatus for the rubber sheeting was not stretched over the animal and the only weight to be moved was that of the writing lever, which is negligible. The rabbit board was placed on a hot tray in order to prevent any fall in the temperature of the animal during the experiment. In some experiments the only further operative procedure was the insertion of a cannula in a branch of the jugular vein, but as a general rule a tracheal cannula was also inserted. This was of the widest calibre that could be employed, and projected not more than 3 cm. from the trachea. The plethysmograph of course precluded any operations on the thorax or abdomen but did not interfere with manipulation of head and neck, so that the blood pressure could be registered and the pneumogastric nerves could be isolated, cut and stimulated without difficulty.

At the end of each experiment the animal was bled, the tracheal cannula was attached to a gas measuring pipette, and the excursion of the lever measured when 5, 10 and 15 cc. of air were driven into the chest. In this way the apparatus was calibrated for each experiment. In the protocols the measurements are given in excursions of the lever without actual reduction to cubic centimeters of air.

I have examined the effects of morphine, chloral, urethane, strychnine and caffeine, using this method.

#### MORPHINE

Gscheidlen (2) appears to be the first experimental investigator of the action of morphine on the respiration, and his statement that the rate of the breathing is much reduced has been confirmed by all succeeding workers on the subject as well as by clinical investigators before him and since. Leichtenstern (3) states that while the slowing of the respiration is the most marked feature, it also becomes shallower in the rabbit. Boeck and Bauer (4) found the carbon dioxide excretion diminished under



morphine in the dog and increased in the cat, and point out that this is accompanied by lessened movement in the former and by spasmodic movements and tremor in the latter. Wood and Cerna (5) injected very large doses of morphine intravenously in the dog and found the respiration arrested immediately. When it was restored by artificial inflation the spontaneous respiration was very slow, but renewed injections of morphine increased it in number and depth, at the same time inducing reflex movements. Filehne (6) had previously described the same phenomenon in the rabbit under large doses (0.05 to 0.1 gram), the respiration being slowed at first and later becoming more frequent in the stage of increased reflex excitability. But even during this stage of increased respiration the excitability of the respiratory center was reduced, for the arterial blood was venous in colour. The lowered excitability of the center was further shown by the ease with which apnoea could be elicited by artificial respiration and by its long duration compared with that in the unpoisoned rabbit. Even after division of the vagi, apnoea could be readily induced in rabbits under morphine, while it was difficult to do so in unpoisoned animals. Filehne and Kionka (7) later showed that the blood in morphine poisoning is poor in oxygen and rich in carbon dioxide, while the respiration is slow, which again indicates lowered susceptibility of the center. And the response to an augmentation of the carbon dioxide (by tetanising the limb muscles) was smaller than in unpoisoned animals. The reaction of the center to carbon dioxide had been examined in man previously by Loewy (8), who concluded that both the strength of the stimulus (carbon dioxide) and the excitability of the center are reduced under morphine. Impens (9) in his advocacy of heroine, made a number of experiments on the effects of morphine on rabbits, from which he infers that morphine reduces the frequency and generally the depth of the respiration, and the response to carbon dioxide inhalation (3 per cent) is entirely lost under it (0.01 to 0.015 gram).

In my experiments on rabbits small doses (1 to 2 mg.) were injected into a branch of the jugular vein at intervals and the rate and size of the excursions were measured two or more min-

utes after each injection. In one respect the results were uniform, the rate of the respiration was slowed, but the extent to which this change developed varied much in different animals, even when the same dose was used. The effect on the excursion of the lever was less constant. In many experiments it remained practically unchanged after a considerable fall in the rate had occurred. In a certain number the movements were distinctly larger after morphine, in a few an increase of 30 to 50 per cent occurring. In very few was there any significant reduction in size, and in these there was evidence of excitement, tremor, or movement before the morphine was injected. The greatest increase in the depth of the respiration occurred in cases in which a single injection caused marked slowing. When an equal degree of slowing was induced more gradually in other experiments by a series of injections the excursions often remained almost unchanged in size throughout. The large change in the rate of the respiration forms a contrast to the comparatively small alteration in the depth (when in fact any occurs). This is illustrated in experiments 1 and 2 and by the chart in figure 2.

The effect of morphine on the reaction to carbonic acid was examined in a number of experiments. For this purpose a bag made of very thin rubber sheeting and holding ten liters was filled with air containing 4.5 per cent carbon dioxide from a cylinder in which the mixture was kept under compression. The bag was closed by a cork with a hole which fitted the tracheal tube, and was quickly adjusted so that the animal inhaled from and exhaled into the bag for about two minutes. After the CO<sub>2</sub> inhalation had lasted one minute, the rate and size of the respirations were measured during the next thirty seconds, as it was found that the rate and depth remained practically constant during the second minute of the inhalation. The bag was refilled for each estimation. In this way the use of valves was avoided as I found that even the lightest valves caused some interference with the respiration. It may be objected that the carbon dioxide in the bag must constantly increase and the oxygen diminish during the inhalation, but as a matter of fact I could not detect a difference of more than 0.1 to 0.2 per cent in the bag after a rabbit had breathed into it for two minutes, and this difference

is negligible. The result of such an estimation before and after morphine is given in experiments 1 and 2.

*Experiment 1. Rabbit of 1600 grams weight, decerebrated under ether. Cannulae in jugular vein and trachea. Carbonic acid was inhaled at intervals in the strength of 4.5 per cent in air. The size and rate of the respiration given under columns 5 and 6 were measured after the inhalation had lasted one minute.*

TIME	FREQUENCY PER MINUTE	EXCURSION IN MILLIMETERS	MORPHINE	CO <sub>2</sub> 4.5 PERCENT	
				Rate	Excursion
15	38	30		50	53
20	35	28		52	52
30	32	30	0.002		
34	28	33		42	55
43	28	33		42	54
50			0.002		
52	24	27		36	52
62	22	30	0.002		
65	19	30		28	52
71	18	31	0.002		
76	17	29		24	53
88	16	32	0.002		
91	14	28		20	51
102	14	32		20	50
112	14	30	0.002		
113	13	30		17	51
134	14	28	0.002		
136	12	28		17	50

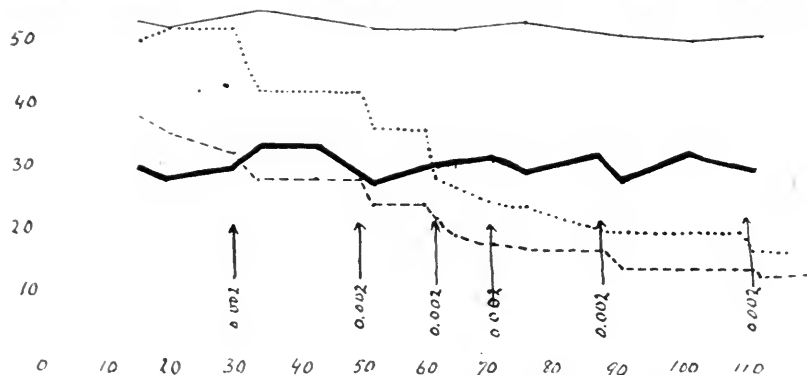


FIG. 2. GRAPHIC REPRESENTATION OF EXPERIMENT 1.

The time is given along the abscissa, the frequency and excursion on the ordinate. Excursion during air breathing——; excursion under CO<sub>2</sub>———; frequency in air-----; frequency in CO<sub>2</sub>.....

*Experiment 2. Rabbit of 1100 grams weight, decerebrated under ether. Cannulae inserted into the jugular vein and trachea. Half an hour later the record was begun. The bag contained 4.3 per cent CO<sub>2</sub>.*

TIME	RATE	DEPTH IN MILLIMETERS	MINUTE VOLUME	DURING CO <sub>2</sub> INHALATION		
				Rate	Depth in millimeters	Minute volume
1	37	16.5	610.5	50	21	1050
6	33	16.0	528.0	48	23	1104
12	38	16.0	Morphine 0.002			
14	22	18.0		33	22	726
21	21	18.5	388.5	34	23	782
26	23	18.5	Morphine 0.002			
28	8 $\frac{1}{3}$	22.0		9 $\frac{1}{3}$	28	261
32	8 $\frac{1}{3}$	24.0	200	10	28	280
38	7 $\frac{1}{2}$	25.0	187	Caffeine 20 mg.		
41	24	19.0	456		23	713

It will be seen that, as has been noted by all previous investigators, the response to carbon dioxide inhalation as measured by the total air breathed is much reduced by the action of morphine; thus in experiment 2 the unpoisoned animal subjected to CO<sub>2</sub> inhalation breathed a total volume corresponding to 1050 to 1104 per minute during the third half minute, while morphine reduced the total volume under CO<sub>2</sub> to 726 to 782 and finally to 260 to 280 per minute. When this minute-volume under carbon dioxide is analysed into its two component factors, rate and volume of individual movements, and these are compared with the rate and volume under carbon dioxide in the unpoisoned animal, these are seen to be very unequally affected by morphine. The volume of each individual respiration remains almost unchanged or may be actually greater than in the normal, while almost the whole fall in the total respiration under carbon dioxide arises from the failure of the center to undergo the acceleration seen in the normal animal. The change in the response to carbon dioxide under morphine is thus parallel to the change observed in air-breathing. In the latter morphine reduces the rate without lessening the depth to a corresponding extent and under carbon dioxide the center cannot respond with an increase in rate while it can still increase in the depth of the movements. The parallelism is well

shown in the chart in figure 2, in which under morphine the depth of the breathing remains almost constant while the rate falls greatly both during air breathing and under carbon dioxide. The same feature appears when the effect of morphine on the  $\text{CO}_2$  response is examined in detail, as in figure 3 from experiment 2, in which the difference in response to carbon dioxide inhalations for two minutes before and after morphine is shown. The change in the depth of the breathing under carbon dioxide is practically the same before and after morphine, while the frequency under carbon dioxide is very much less after the injection.

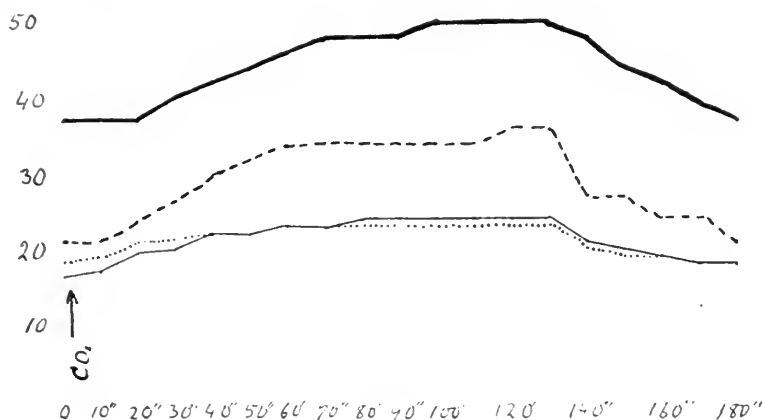


FIG. 3. CHART OF THE FREQUENCY AND DEPTH OF RESPIRATION UNDER  $\text{CO}_2$  INHALATION BEFORE AND AFTER MORPHINE

Time in seconds on the abscissa. Frequency and depth on the ordinate.  $\text{CO}_2$  was inhaled for 120 seconds; frequency before morphine——; frequency after morphine-----; depth before morphine——; depth after morphine..... From experiment 2 at six minutes (before morphine) and at twenty-one minutes (after morphine).

It has long been known that section of the vagi induces a very slow and deep form of respiration, and Scott (10) showed some years ago that after division of the vagi in the neck, carbon dioxide inhalation caused the usual increase in the depth of the movement but was no longer followed by any significant acceleration. Carbonic acid thus seems to act on the respiratory center directly in augmenting the depth, but can accelerate only as long

as the center receives impulses from the lungs through the afferent nervous paths. This similarity in the effects of vagus section and morphine suggested that the latter may act on the respiratory center by blocking the passage through the synapse of afferent impulses to the respiratory center. This would also explain the effect of morphine in cough. Several experiments were therefore performed on rabbits with divided vagi, but the effect on the respiration resembled that seen in experiments in which the nerves were intact, except that after division of the vagi morphine increased the depth of the respirations more frequently and to a greater extent. The fatal dose of morphine for rabbits was much reduced when the vagi were cut. Gscheidlen noted that the effects of morphine on the rate of respiration were greater in these animals.

*Experiment 3. A rabbit of 1000 grams weight was decerebrated under ether, and cannulae were put in the trachea and jugular vein. Both vagi and one superior laryngeal nerve were tied. Respiration recorded as described. Seventy-five minutes after the vagi were divided the following observations were made.*

TIME	RATE OF RESPIRATION PER MINUTE	EXCURSIONS OF LEVER IN MILLIMETERS	INTRAVENOUS INJECTION OF MORPHINE
75	26	39	0.002
76			
80	18	32	
82	15	38	0.002
83			
85	15	50	
89	13	51	0.002
101			
103	11	50	
105			0.002
107	7	53	0.002
109			
113	6	58	
120	Spasmodic	movements	

In one experiment morphine was injected until the respiration was much slowed and both vagi were then cut. This was followed at once by a very marked fall in the rate, which did not differ in degree from that seen on cutting the vagi in the unpoisoned animal.

From these experiments it is evident that morphine does not lessen the rate merely by preventing the access of impulses to the center from the lungs.

The same conclusion may be drawn from experiments in which the central end of the vagus was stimulated with series of induction shocks. The effect of central vagus stimulation has been the subject of much discussion, of which the general result is that while weak shocks generally cause slowing and weakening in the position of expiration, stronger currents cause acceleration with imperfect expiration or even complete standstill in the position of full inspiration. In my experiments both vagi and both superior laryngeal nerves were ligated. The vagus was stimulated by covered electrodes which were placed as far down the neck as possible in order to avoid any possible escape of current to the superior laryngeal nerves; the fact that the weaker shocks caused inhibition while the stronger ones, which would naturally be more liable to affect the superior laryngeal, were followed by acceleration, shows that the vagus impulse was elicited independently of the laryngeal one.

In several of the earlier experiments in which morphine was gradually injected and the vagus stimulated at intervals with the minimal current that caused acceleration at first, the stimulation slowly became less effective and finally induced inhibition. This was interpreted as indicating that morphine depresses the synapse in the accelerator afferent path while leaving that of the inhibitory path unaffected. But it soon appeared that the same decline in the efficiency of the vagus accelerator fibers occurs in a long experiment without any drug having been injected; in other words, the augmentor reflex is more readily fatigued than the inhibitory one, and the apparent selective action of morphine is due to this gradual fatigue. A comparatively slight increase in the strength of stimulus was often sufficient to demonstrate that the change in the reflex excitability had only changed to a small extent however.

In later experiments the inspiratory reflex remained unchanged under very large doses of morphine which reduced the rate of the respiration greatly (fig. 4).

The absence of any change in the inspiratory reflex in these later experiments must carry greater weight than the apparent diminution in the earlier ones, and the inspiratory reflex from the vagus thus seems to be unimpaired by quantities of morphine which are sufficient to reduce the rate of the breathing to a very marked extent.

Similarly, the expiratory reflex from stimulation of the vagus with weaker currents presented no diminution after morphine. In unpoisoned animals weak shocks often cause arrest of the respiration, which after some seconds is interrupted by slow and

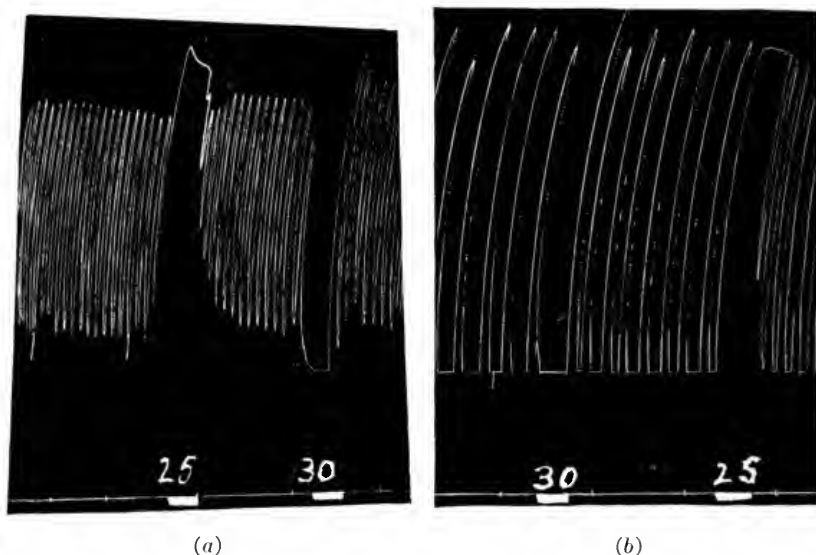


FIG. 4. RESPIRATION UNDER VAGUS STIMULATION AT 25 AND 30 CM. DISTANCE BETWEEN THE COILS

*a*, Before morphine; *b*, thirty minutes later when 8 mg. of morphine had been injected. In each case stimulation at 25 cm. causes inspiratory standstill, and at 30 cm. inhibitory standstill in the expiratory position.

weak movements. There was less tendency for this interruption to occur after morphine in some experiments, and in others the arrest lasted after the stimulation ceased, while in the normal the breathing commenced at once. But in most cases no distinct alteration of the character of the response could be determined;



and the minimal strength of stimulus remained unchanged by morphine (fig. 4). The inhibitory vagus reflex is certainly not weakened by morphine and sometimes is more effective than before the injection.

The reflex from the superior laryngeal was intensified under morphine. A weaker stimulus was sufficient to cause distinct slowing than before morphine and a strength of stimulus which previously caused only slight slowing now gave a more distinct effect or caused complete arrest (fig. 5).

The same result was obtained when the laryngeal reflex was elicited by chemical irritation of the mucous membrane with ammonia vapour. For this purpose 50 cc. of a  $\frac{N}{3}$  solution of ammonia was put in a liter aspirator bottle. A tube was carried from the mouth of the bottle to a glass cannula which was passed into the larynx from the opening in the trachea and a second rubber tube led from the opening in the side of the bottle to an empty 10 cc. syringe. When the air in the syringe was discharged into the bottle, a puff of ammonia vapour was carried to the larynx and induced inhibition of the respiration along with repeated swallowing movements. The method is not exact, but from a number of observations it appeared clear that more complete inhibition was obtained after morphine than before it, the standstill lasting considerably longer in the poisoned animal.

In the rabbit, irritation of the larynx or stimulation of the superior laryngeal nerve causes only inhibition of the respiration. There is nothing corresponding to the fixation of the thorax and the abdominal compression of true cough. In the cat these are elicited by stimulation of the superior laryngeal and by ammonia vapour in the larynx, but not by any means regularly, and I have not been able to investigate whether morphine has any special effect on the active phase of cough therefore.

The result of these experiments on the afferent fibers of the respiratory reflexes indicates that morphine has little or no effect on the augmentor impulses, while it renders the inhibitory impulses of the vagus, and more especially those from the superior laryngeal, more effective. This does not indicate definitely that it renders the synapse on the inhibitory path more permeable.

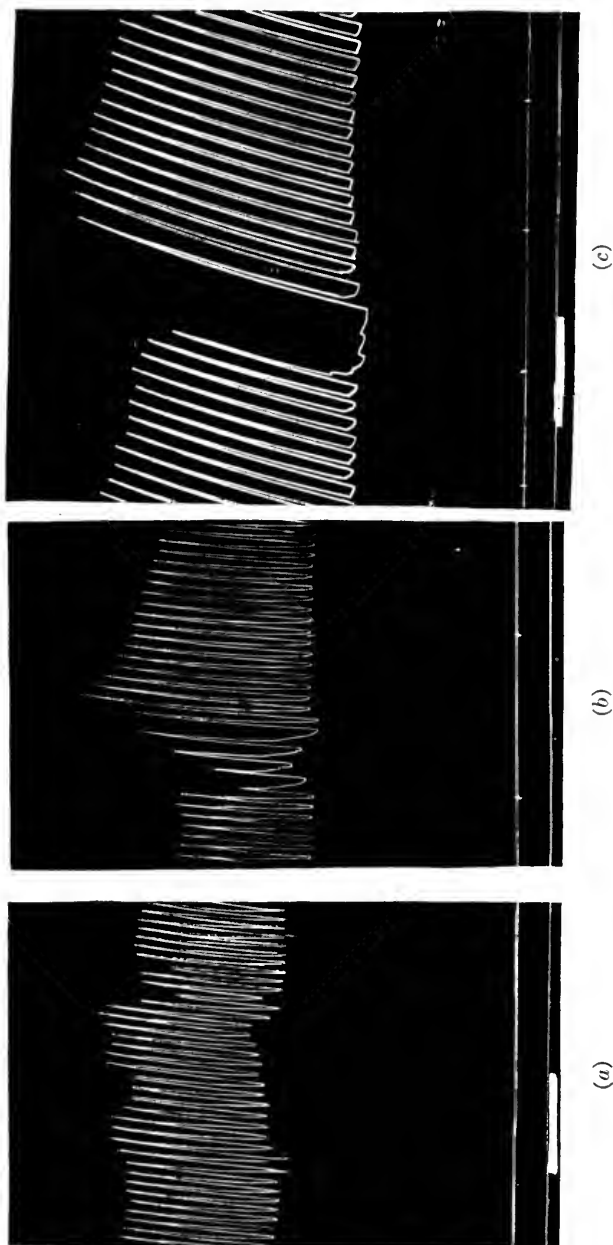


FIG. 5. RESPIRATION UNDER STIMULATION OF THE SUPERIOR LARYNGEAL AT 27 CM. DISTANCE BETWEEN THE COILS  
*a*, Before morphine; *b*, after 0.001 morphine; *c* after 0.004 morphine. Time in half minutes.

however, for this more effective inhibition is only seen when the respiration is considerably slowed, that is, when the motor center itself is emitting fewer impulses. And it is therefore probable that the inhibition is more effective because it is acting on a more sluggish organ. In the same way the apnoea from forced breathing lasts longer under morphine (Filehne).

An analogy may be drawn with the response of the heart to inhibitory impulses, which have been found more effective after poisoning with potassium (Howell (11) Fleischhauer (12)), though potassium does not act on the inhibitory mechanism but on the muscle directly. In the same way the greater effectiveness of inhibitory stimulation on the respiratory center under morphine is probably due to the depression of the center, of which there is abundant evidence, rather than to the inhibitory impulses reaching the motor cells more easily. In other words, the change in the reflex response follows from the slowing.

These experiments indicate that while morphine induces changes in the respiration which resemble those following division of the vagi, more especially as regards the response to carbon dioxide, the alkaloid does not act through reducing the susceptibility of the center to afferent stimuli, for the augmentor reflex is not appreciably altered, while the inhibitory is actually more effective. And these changes may be regarded as a secondary result of the action on the center and not as the prime factor. As regards the other influences which act upon the respiratory center, the response to carbonic acid is changed to a marked extent by morphine, and this change in the response to carbon dioxide runs exactly parallel to the change in the air breathing before and after morphine. Only one side of the respiration is affected however, for while the frequency is much reduced, the depth remains unchanged or may be augmented. And stimulation of the center under morphine by excess of carbon dioxide similarly affects the depth mainly, while the rate is much less accelerated by carbon dioxide than in the unpoisoned animal. It is true that some increase in frequency is induced even at the end of my experiments by the inhalation of carbon dioxide, but this is very small compared with that induced before morphine,

while the depth attained at this time under carbon dioxide was as great as or greater than that observed at the beginning of the experiment.

The rabbit under morphine exhibits less active movement than before it, and this in itself must reduce the  $\text{CO}_2$  production and the strength of the respiratory stimulus. The slight diminution in the depth of the breathing which has been noted by some observers may in fact arise from this acapnia. On the other hand, this is met by the slowing of the respiration, which would in itself tend to increase the carbon dioxide in the blood. When these two factors are equivalent, the depth of the respiration remains unchanged. If the slowing is more developed than the general narcosis and the carbon dioxide thus accumulates in larger quantity in the blood, the respiratory center is stimulated and, unable to respond by quickening, can only cause deeper breathing. The depth of the respiration may thus increase under morphine, but this is secondary to the lessened frequency, and indicates that the carbon dioxide of the blood is increased. The great deepening of the respiration is thus seen at the stage of greatest slowing.

The principles of the respiratory regulation were fully realised a number of years ago, and the view which I have given above is in accord with the older observers. Confusion was brought into the subject by the work of Dreser (13) and Impens (9), who stated that while morphine has comparatively slight effects on the depth, diacetylmorphine has an apparently specific action in deepening the respiration. In their experiments however they fail to recognise that the doses of heroine used caused a very marked slowing of the respiration and that the increased depth is merely a reaction to the accumulating carbon dioxide. If the dose of morphine given had reduced the respiration as much in rate, the depth would have been correspondingly increased. Under heroine, as under morphine, the depth of the respiration is determined by the frequency and the carbon dioxide production. Dreser states that under heroine the rate is diminished but that partial asphyxia increases the depth as much as before

the injection, and he concludes from this that the susceptibility of the center to carbon dioxide and to oxygen lack remains unchanged under heroine. But he failed to appreciate that while the response to carbon dioxide is unchanged in depth the frequency is much reduced; that is, the effect of heroine on the center is precisely that which I have noted under morphine.

Morphine did not alter the response to reflex excitation of the respiratory center in the rabbit except indirectly through the change in the frequency. The methods of investigation were not strictly comparable to the conditions in cough, for in the latter there is a slight prolonged excitation which finally culminates in cough, while in my experiments a sudden irritation was induced. It is possible that under morphine the excitability by minimal long continued irritation may be reduced, while a sudden stimulus may be able to arouse the center to activity. This is supported by the fact that a slowly increasing stimulus under morphine fails to cause the sensation of pain, while a sudden stimulus of rather less intensity may do so. But it is possible to explain the lessened tendency to cough under morphine by the lessened activity of the center without resort to the view that the afferent impulses are weakened. For the reduced activity of the respiratory center renders it more susceptible to inhibitory impulses, and the relief of cough may thus be the result of the slowing action on the respiration alone.

Two experiments done on cats have not been considered in the account of morphine action on the respiration. It may be stated however that 1 to 2 mg. of morphine act on the respiration of decerebrated cats in the same way as in decerebrated rabbits and on the whole appear to depress it more strongly. No evidence of stimulation of the respiration was seen, and it seems probable that the excitement generally seen in cats is cerebral in origin (if it is not asphyxial). I have not observed the second stage seen by Filehne in rabbits in which the respiration became more rapid again; this may arise from my rabbits having been decerebrated and the doses smaller.

## CHLORAL HYDRATE AND URETHANE

The effects of chloral hydrate on the respiration have been less frequently examined than those of morphine. Ordinary therapeutic doses of chloral are generally believed to have little effect on the respiration except in so far as they cause sleep and thus render the breathing shallower and slower, as in natural sleep. Loewy (8) showed that in man even large doses (4 grams) caused no material change in the excitability of the center by increasing concentrations of carbon dioxide, the alteration in response not being greater than that in natural sleep; the reduced ventilation under chloral arises from the lessened production of  $\text{CO}_2$  from the diminished movement and not from the excitability of the respiratory center falling. Wood and Cerna (5) found in the dog that 0.5 to 2 grams reduced the minute volume very considerably. From a calculation of their figures it would appear that this was in some cases due to a fall in the rate only, the depth remaining unchanged. In others the depth must have been reduced without material change in the rate. But the dogs were examined without previous elimination of the cerebrum, and this reduces the value of the observations since ventilation may probably have been excessive in the commencement from the excitement of the animal. The effects of chloral on the respiratory reflexes was the subject of some discussion, Burkart maintaining that vagus stimulation after chloral causes the inspiratory reflex only, Rosenthal holding that both expiratory and inspiratory reflexes are lost, and Fredericq (14) and others finding that the effect varies with the dose but that in the deepest stage of chloral anaesthesia the expiratory reflex alone survives. Langlois and Richet (15) state that chloral in large doses prevents reinforced expiratory movements, and animals in which the respiratory passages are narrowed die from failing to overcome the resistance to expiration, but they attribute this rather to the narcosis of higher nervous functions than those of the respiratory center.

- I have made a number of experiments with chloral with the object of comparing its effects on the respiration with those of morphine. The protocol of one of these shows the results ob-

tained in all. The chloral was injected in 5 per cent solution into a branch of the internal jugular vein. Occasionally, when the injection was made too rapidly, the respiration was accelerated for some time, obviously from the action on the heart lowering the blood pressure and inducing anaemia of the brain.

*Experiment 4.* A rabbit of 1700 grams weight was decerebrated under ether, and cannulae put in the trachea and the anterior branch of the jugular vein. The respiration was recorded in the usual way and carbon dioxide (4.5 per cent) was supplied for two minutes at intervals. The measurements under the rubric CO<sub>2</sub> were taken between one minute and one minute thirty seconds after the beginning of the inhalation. The CO<sub>2</sub> inhalation was begun only 3-4 minutes after the injection.

TIME IN MINUTES	RATE PER MINUTE	EXCUR- SIONS IN MILLI- METERS	CHLORAL	RATE AFTER DRUG	EXCUR- SIONS AFTER DRUG	EFFECT OF CO <sub>2</sub>	
						On rate	Depth in millimeters
1 - 3	46	24				74	43
5 - 8	54	24				74	44
16 - 21	52	23	0.04	48	23	76	48
23 - 29	54	22	0.05	46	23	70	49
33 - 38	45	26	0.05	49	24	71	48
42 - 48	44	25	0.05	44	28	64	48
50 - 55	45	28	0.05	42	28	62	48
62 - 64	43	31				66	46
68 - 72	46	33	0.05	49	31	62	44
75 - 80	46	37	0.10	41	32	51	39
86 - 88	32	37				48	39
91 - 96	36	36	0.10	32	32	46	37
97 - 104	34	36	0.20	22	36	28	38
107 - 109	5	56				8	55

In the first seventy-two minutes are shown the effects of 0.17 gram chloral per kilo injected intravenously. The rate is practically unchanged, while the depth is increased from 24 to 33. The response to carbon dioxide is perceptibly altered, the rate of the respiration under it being slightly less, while the excursion is somewhat greater than in the beginning. In the latter part of the experiment (75 to 104 minutes) larger quantities (0.28 gram per kilogram) cause a distinct fall in the rate, and still later, when 0.4 gram per kilogram had been given, a sudden fall in the rate of the respiration occurs, while the depth increases greatly and marked forced breathing sets in. This sudden change in

the respiration late in chloral poisoning was observed in other experiments in which the later injections had been more gradual than in experiment 4.

In this experiment the respiration under air breathing is but little affected by chloral up to 0.17 per kilogram, but that the center is less capable than before is shown by the response to carbon dioxide, under which the acceleration is much smaller than before the drug was given. The depth of the breathing in air increases under this dose and also under carbon dioxide, though to a less extent. Under chloral in this amount the reflex movements and general activity are very much diminished and the  $\text{CO}_2$  production must fall in a corresponding measure. The respiratory stimulus would therefore appear to be diminished. Yet the ventilation in air is increased, for the excursion is larger with a constant rate, and this might suggest that the excitability of the center is actually increased by chloral. This is not the inevitable inference however, for the effect of chloral on the circulation must be taken into account. In weakening the heart chloral must necessarily retard the medullary blood supply, and this would cause the same effect as an increased  $\text{CO}_2$  tension. The increase in the depth of the breathing is thus evidence of an increase in the stimulus of the respiration, even though the narcosis may reduce the actual total  $\text{CO}_2$  production. The center is however less able to respond to the stimulus by acceleration than in the normal, for under  $\text{CO}_2$  inhalation the acceleration is smaller than before the injection. The breathing is deeper under chloral while the rate remains unchanged, because although the  $\text{CO}_2$  production in the muscles may be reduced by the narcosis, the  $\text{CO}_2$  stimulus in the respiratory center is reinforced by the weakness of the circulation. This reinforced stimulus also explains the fact that the frequency in air breathing is little changed in the earlier part of the experiment, while the response under  $\text{CO}_2$  shows that the excitability is lowered. A constant rate of respiration does not indicate an unchanged excitability of the center unless both the carbon dioxide and the circulation in the medulla remain unchanged. Here both the carbon dioxide and the excitability are undoubtedly lower but the failure of the



circulation counteracts the effects which would naturally result from these changes.

As the dose is increased the excitability of the nerve center is so far reduced that it can no longer maintain the rate even under the double stimulus of carbon dioxide and anaemia, but it continues to deepen. Finally its rate is reduced to one-ninth of the normal, while its depth is doubled.

The same general effect was obtained from chloral in animals whose vagi had been divided previously, the rate falling under large doses while the depth increased.

Three experiments were performed with urethane in rabbits, of which I give one example. The urethane solution has to be diluted to about 2 to 5 per cent for intravenous injection, as stronger solutions often cause marked acceleration of the respiration lasting for some minutes. This is obviously due to a "salt-action" from the concentrated liquid, for the same acceleration follows the injection of strong salt solution.

In experiment 5 (p. 384) the depth of the respiration showed little variation until the rate was reduced very considerably, when the depth increased. A lower response to carbon dioxide is seen earlier showing a reduced excitability of the center. Urethane has very little action on the circulation, as is generally recognised, and the  $\text{CO}_2$  stimulus is thus not reinforced by anaemia as under chloral, so that in air breathing the depth is not altered until the rate was seriously impaired.

Several times in this experiment (at 42, 68 and 78) the  $\text{CO}_2$  inhalation slowed the respiration instead of accelerating it, while the depth was increased. I have observed the same curious reaction under chloral in two instances. It seems unlikely that the dilution of 4.3 per cent  $\text{CO}_2$  could have acted as a narcotic to the center, but I am unable to suggest any other explanation; it is possible that under these large quantities of narcotics the  $\text{CO}_2$  may acquire this power by a species of synergism. Scott (10) states that in rabbits with divided vagi  $\text{CO}_2$  over 6 to 7 per cent generally slows the respiration. It is to be remarked that he used chloral as an anaesthetic and that in my experi-

*Experiment 5. Rabbit of 1215 grams weight was decerebrated under ether, and cannulae were placed in the trachea and the jugular vein. CO<sub>2</sub> (4.3 per cent) was inhaled at intervals for two minutes.*

TIME	FREQUENCY	DEPTH IN MILLIMETERS	UNDER CO <sub>2</sub>	
			Frequency	Depth in millimeters
1	44	21	68	30
7	44	19	64	31
14	Urethane 0.25			
16	40	17½		
18	38	20		
19	Urethane 0.25			
21	32	20	46	32
24			48	34
30	34	19		
31	Urethane 0.5			
35	30	20		
37	Urethane 0.5*			
42	25	25	21	32
49	28	22	30	30
55	27	22	34	27
62	27	20†		
68	26	22	23	26
78	23	24	20	30

\* Respiration ceased after four breaths rapidly diminishing in depth. Artificial respiration was instituted. After a few minutes each inflation was followed by a spontaneous respiration, but when the artificial respiration was interrupted no spontaneous respiration occurred. Artificial respiration resumed, the interpolated spontaneous movements soon returned, and when the artificial inflation ceased spontaneous breathing continued.

† Urethane slowed and then arrested the respiration which was restored by artificial respiration, the phases resembling those seen before (see figure 6).

ments large quantities of these narcotics caused the same reversal of the CO<sub>2</sub> effect when the nerves were uninjured.

During the artificial respiration, each inflation was followed by a spontaneous respiration, but when the artificial respiration was interrupted, no spontaneous movement followed. The center was thus susceptible to the reflex impulse arising from inflation at a time at which it was devoid of its rhythmical power (see fig. 6). This is in accord with the results of stimulation of the central end of the vagus, which under chloral and urethane gave

generally the normal results. In two experiments in which vagus stimulation at first gave a mixed response partly inhibitor and partly accelerator, the effect changed to pure inhibition under repeated injections of chloral, but this may have been due to fatigue of the augmentor fibers, and not to the drug. In other instances no definite reduction in the augmentor reflex occurred, while the inhibitory reflex seemed rather more effective, as has been described under morphine.

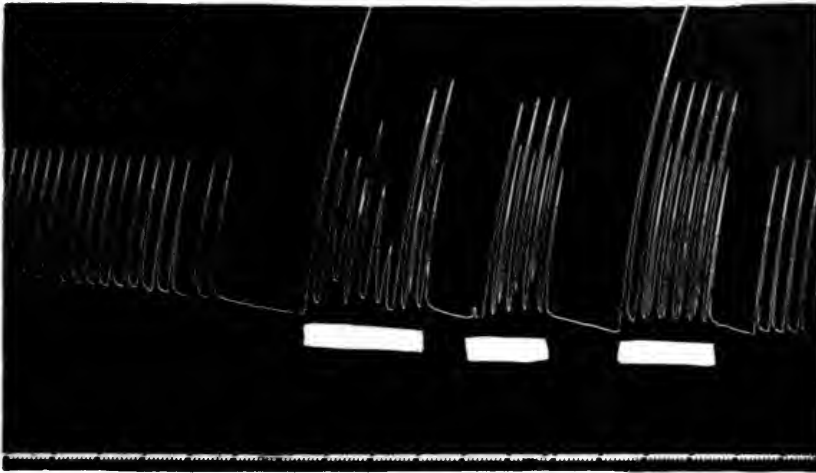


FIG. 6 FAILURE OF THE RESPIRATION UNDER URETHANE

Resuscitation by artificial respiration, which is indicated by the underlined parts of the tracing. Time in one second intervals. The artificial inflations were given at the rate of twenty-four per minute.

#### CAFFEINE

Caffeine has been largely used in therapeutics as a stimulant to the respiration, chiefly in the form of hot coffee in opium poisoning. The pure substance has been used experimentally by Binz (16) who recorded the respirations by means of a tambour connected with the T-shaped tracheal cannula, and found the breathing quickened and deepened by caffeine in a dog. Heinz (17) found the minute volume in the rabbit increased by small quantities (0.005 to 0.010), but not by larger ones. Impens (18),

using Dreser's method, found the rate of respiration slightly increased in the rabbit by 0.02 gram but this effect is not constant. The minute volume is also increased generally while the individual movements are sometimes increased but oftener diminished. The same result is obtained from 0.05 gram which induces restlessness, while still larger induce convulsions, which are of course attended by increased ventilation.

In my experiments in which caffeine was injected intravenously in rabbits in quantities of 0.01 to 0.02 per kilo, the first effect was often a marked increase in tone, the expiratory position moving

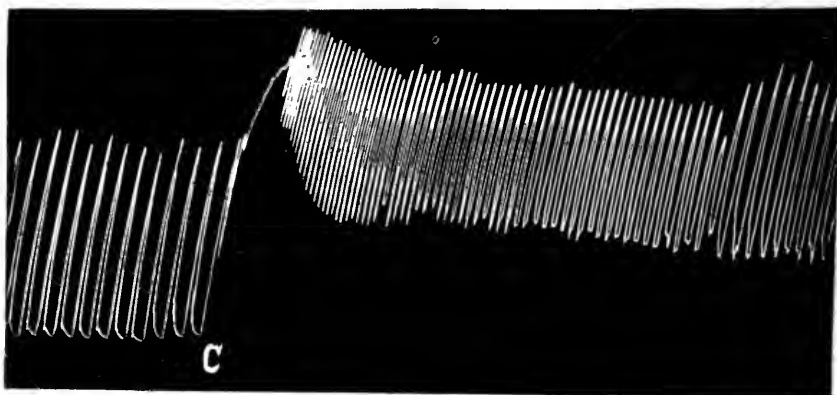


FIG. 7. RESPIRATION UNDER MORPHINE

At *C* intravenous injection of 10 mg. caffeine. The vagi had been divided early in the experiment.

up from the base line very distinctly (fig. 7). If the injection was made suddenly this often culminated in a short inspiratory spasm from which recovery followed by a series of rapid movements in each of which the expiration was more complete until it reached a position a little above the original expiratory position. At this time the respiration was much accelerated, and it continued more rapid than the normal for a varying time. When the injection was made more slowly, the respiration was generally accelerated to a considerable degree, but in a few instances this acceleration was not marked, and in one instance caffeine actually slowed the respiration.

The depth of the respiration was comparatively little changed by caffeine during the acceleration. On the whole it was rather shallower than previously, but in many cases the difference was not more than 1 to 2 mm. When spasm was induced by caffeine the depth was much increased and even comparatively slight movements of the limbs, such as occurred not infrequently, caused some deepening. When these were absent, however, no

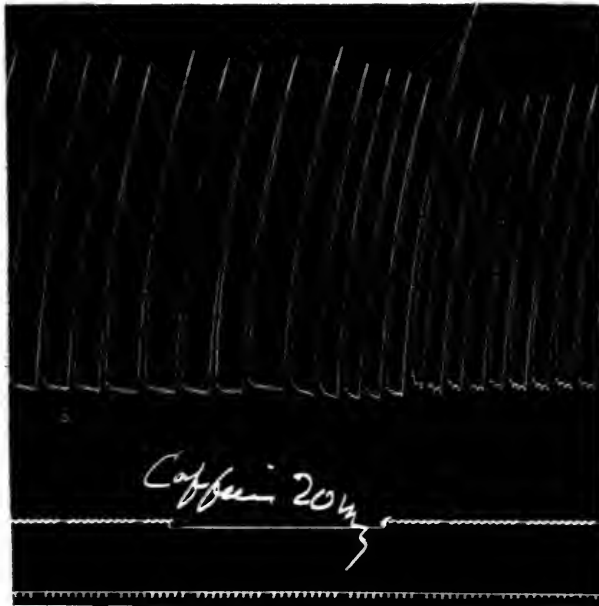


FIG. 8. RESPIRATION TRACING UNDER LARGE DOSE OF CHLORAL

The injection of caffeine (0.02 gram) accelerates the movements and reduces them in size.

material change in the size of the respiratory movements were observed.

When the respiration had been slowed previously by morphine or chloral, the acceleration was equally observable under caffeine (fig. 8), and as spasm was less frequently induced, the acceleration uncomplicated by change in depth was often clearer.

*Experiment 6. Rabbit of 1250 grams, decerebrated under ether. Cannulae in the trachea and jugular vein*

TIME	FREQUENCY	DEPTH IN MILLIMETERS	DURING CO <sub>2</sub> INHALATION	
			Frequency	Depth in millimeters
2	46	23	80	38
8	48	20	74	41
15	48	20	Caffeine 0.02*	
19	94	31	132	42
24	106	22	128	42
31	69	21	110	45
36	64	22		

\* Caused great rise in the respiratory position culminating in spasms lasting for 45 seconds.

In this experiment the acceleration was the highest observed. The depth remains practically unchanged except at nineteen minutes when it is much deepened. But some restless movement was observed in the limbs at this time and the increase in depth may fairly be attributed to it.

*Experiment 7. Rabbit of 1600 grams, decerebrated under ether. Cannulae in trachea and jugular vein. In the course of two hours 0.014 morphine was injected intravenously and the respiration fell to 12 per minute.*

TIME	FREQUENCY	DEPTH IN MILLIMETERS	UNDER CO <sub>2</sub>	
			Frequency	Depth in millimeters
140	12	28	17	42
146		Caffeine 0.02 grams injected		
151	21	27	24	43
161	14	30	Caffeine 0.04	
166	54	30		

In most instances the action of caffeine was only transient, the effect passing off in five to ten minutes. Experiment 6 is quite exceptional in the duration of the acceleration.

A second injection had often much less effect than the first one on the frequency of the respiration and occasionally was followed by no change or even by slowing. The reaction to carbonic acid was examined in a number of instances. During

the acceleration the effect of carbon dioxide was much increased as is seen in the protocols of experiments 6 and 7. Thus a strength of carbon dioxide in experiment 6 which before caffeine induced a rhythm of 74 to 80, afterwards caused an acceleration to 130 per minute. But when the rate under  $\text{CO}_2$  is compared with that under air, it is found that the two bear a ratio which is not very distant from that obtained by a corresponding comparison before caffeine.

The depth of the respiratory movements under carbon dioxide is approximately the same before and after caffeine. Thus in experiment 6 before caffeine the depth was 38 to 41 mm. under  $\text{CO}_2$  inhalation. Afterwards it was 42 at the highest acceleration, rising to 45 as the acceleration fell off.

Caffeine thus acts almost exclusively on the rate of the respiration, while it has little or no influence on the depth of the movement, and the highest acceleration induced by it may be accompanied by no change or even by diminution in the extent of movement.

In unpoisoned animals carbon dioxide sensibly accelerates the respiration only when the vagi are intact. Caffeine however differs from the hormone in the fact that section of the vagi does not appear to prevent the acceleration (see fig. 7). The action of caffeine on the respiratory center is thus different from that of carbon dioxide, the specific stimulus.

Again the excitation induced by caffeine cannot replace that carried by the afferent nerves. For carbon dioxide fails to accelerate the respiration when the vagi are divided, even when the rate has been restored by caffeine to that prevailing before the section or even beyond it.

During the acceleration stimulation of the superior laryngeal was less effective than before caffeine was given. This was shown in the inhibitory pause of the respiration being interrupted by movements which were slower than those preceding the stimulation. In other cases the inspirations were weakened but scarcely slower under caffeine when the superior laryngeal was stimulated with a strength of current which had arrested the respiration before caffeine was injected.

The inhibitory effect of the vagus was similarly affected. In some experiments the action on the vagus reflex showed itself simply by the pause being interrupted by spontaneous respirations during the stimulation, while the inhibitory pause was unbroken before caffeine was injected. In other instances stimulation of the vagus at a coil distance which previously caused pure inhibition now induced acceleration or inspiratory standstill. A weaker current now caused pure inhibition again. Here there is a true reversal of the reflex which is similar to that known to occur in the limb muscles of the frog under strychnine and which has been studied by Sherrington in the mammal. This reversal did not occur in my experiments on the superior laryngeal reflex but only in those on the vagus in which the accelerator and inhibitory fibers are both strongly represented. Sherrington was at first inclined to regard the reversal as indicating that an inhibitory impulse was actually changed in character to an augmentor one, but this view seems less satisfactory than another, that the inhibitory impulses pass to the motor neuron as usual but fail to affect it because it receives at the same time much reinforced augmentor impulses. In reversal then, the character of the impulses reaching the center is unchanged, but the balance between the augmentor and inhibitory impulses is displaced in favour of the former. This view is borne out in my experience of the respiratory reflexes, for the expiratory (inhibitory) reflexes remain in force under caffeine and may be elicited as in the unpoisoned animal if the secondary coil be pushed further from the primary. The augmentor and inhibitory reflexes are both aroused by stimulation of the vagus in the normal, but the inhibitory being more easily excited, a weak current manifests only inhibition. If however the balance is displaced by caffeine so that the augmentor reflex is strengthened, stimulation now reveals the augmentation which was previously latent, while the inhibition which was formerly dominant disappears. On weakening the stimulus again, the advantage which the caffeine gives the augmentor reflex disappears, owing to the augmentor fibers being less excitable, and the inhibitory impulses once more regain control.



This change in the power of the augmentor reflex may arise either from caffeine rendering the synapse of the afferent fibers more readily traversed by the impulses or by the emitting nerve cells being rendered more readily excitable and thus more susceptible to those impulses. The latter view seems to be the more plausible, because the center is also more susceptible to carbon dioxide, as I have shown. It seems unnecessary therefore to assume that caffeine acts on the sensory synapses, unless it be also accepted that carbon dioxide acts through these, for which there seems no foundation. The increased activity of the motor cells is also seen in the inhibitory pause from superior laryngeal stimulation being broken through by respiratory movements.

It may be added that carbon dioxide has a very similar effect on the inhibition of the respiration, for in the unpoisoned animal during the inhalation of carbon dioxide the respiration is no longer arrested by stimulation of the superior laryngeal at a coil distance which is sufficient when air is breathed. In this case the presence of the stimulus, carbon dioxide, opposes the inhibitory reflexes, while under caffeine the greater excitability of the center has the same result.

#### STRYCHNINE

The effects of strychnine on the respiration have been examined by Wood and Cerna (5), Impens (18) and Biberfeld (19). Wood and Cerna, experimenting on dogs, found that the subcutaneous injection of strychnine (0.0005 to 0.001 gram) increases the rate and also the depth of respiration, especially when it has been previously reduced by chloral. Impens found that in rabbits strychnine had no distinct influence under 0.38 mg. per kilogram; this was sufficient to increase the rate and depth of the respirations but only for a short time. Biberfeld states that 0.1 mg. strychnine increases the depth of the rabbit's respiration but not constantly. More definite results were obtained on animals previously narcotised with morphine, in which both the rate and the depth appear to be increased.

In experimenting with strychnine I have found a great difficulty in the occurrence of spasms or of slight tremors. The first

are unmistakable, but it is often difficult to determine whether there may not be slight tremor present. A constant effect following the injection of 0.1 mg. intravenously is a slight rise in the level of the expiratory excursions, which apparently are not so complete as before. This might indicate either an increased tone of the respiratory muscles which check before the full expiratory position is reached, or a slight tonic contraction of the back muscles, which raises the trunk from the board and thrusts it more completely into the plethysmograph. In either case there is a distinct tonic contraction of some groups of muscles, which must complicate the effect of strychnine on the respiratory center by adding to the direct action of the alkaloid that of an increase in the  $\text{CO}_2$  tension of the blood. This indirect action through the formation of carbon dioxide is much more developed when weak tremor is induced by strychnine and of course is extreme when an actual spasm is precipitated. It is thus necessary to consider in apparent stimulation how far the results may be ascribed to the increase in  $\text{CO}_2$  production from increased contraction or tremor of the general musculature. In the experiments on rabbits and one cat the small dose of strychnine (0.05 to 0.1 mg.) was not followed by any obvious movement except the small upward movement of the tracing already mentioned. The respiration was accelerated but not deepened appreciably and was often shallower than before the drug was injected. The acceleration was not very great from these small doses, but appeared more persistent than that from caffeine. In several experiments, the rabbits were anaesthetised with urethane instead of being decerebrated, as it was found that large doses could be used under anaesthesia with less tendency to spasm and tremor than was met under smaller amounts in decerebrated animals.

In experiment 8 the amount of strychnine tolerated was very large from the previous administration of urethane. The same results were observed in decerebrated rabbits from about one-tenth of the dose of strychnine. Strychnine accelerated the respiration and rendered it more shallow as long as no spasms were present. But when the injection caused spasm as in this experiment at 32 the depth increased as well as the frequency.

*Experiment 8. Rabbit of 1400 grams, narcotised with 2.0 grams urethane subcutaneously. Cannula in the jugular vein; the right superior laryngeal nerve tied and put on electrodes.*

TIME IN MINUTES	RATE PER MINUTE	DEPTH
17	34.	27
17½	0.5 mg. strychnine	
19	44	21
22	38	20
23	1.0 mg. strychnine	
24	44	18
30	48	21
	1 mg. strychnine	
31	50	18
32	2 slight spasms	
33	64	24

The action of strychnine appeared to be direct on the respiratory center, for if it had arisen mainly from increased CO<sub>2</sub> production, the depth would have increased along with the rate. This was shown definitely however in a series of experiments in which the vagi were divided before the injection; for if the acceleration from strychnine were secondary to the CO<sub>2</sub> production it would not occur after vagus section. As a matter of fact the acceleration was more pronounced after section of the vagus than in animals in which the nerves were intact.

*Experiment 9. Rabbit of 1200 grams weight, decerebrated under ether, and cannulae put in the trachea and jugular vein. Both superior laryngeal and both vagi tied off. Twenty minutes later the record began.*

TIME	FREQUENCY PER MINUTE	DEPTH IN MILLIMETERS
24	24	18
24½	Strychnine 0.1 mg.	
26	34	16
36	32	17

When the vagi were divided and amounts of strychnine large enough to cause spasm were injected, the acceleration became extreme.

There can therefore be no question that strychnine increases the excitability of the respiratory center directly, though this

direct action may be reinforced by the increased production of  $\text{CO}_2$  from the muscle activity.

Under strychnine the inhalation of carbon dioxide (4.3 per cent) was followed by greater acceleration than previously, while the size of the excursions did not show any greater augmentation than before the injection.

*Experiment 10. Decerebrated rabbit of 1150 grams weight with cannulae in trachea and jugular vein*

TIME IN MINUTES	FREQUENCY	DEPTH	UNDER $\text{CO}_2$ INHALATION	
			Frequency	Depth
15	56	16	72	26
20	54	$16\frac{1}{2}$	72	26
29		Strychnine 0.05 mg.		
32	62	16	80	26
37	66	14	80	28

The reaction to increased carbon dioxide under strychnine is thus parallel to the reaction in air breathing. On comparing the respiration under carbon dioxide before and after strychnine the acceleration is seen to be greater after the injection while the depth is little altered.

The respiratory reflexes under strychnine have been examined by Seemann (20) since I commenced my experiments. He finds that the reflex arrest of respiration in the expiratory position, which follows the application of ammonia vapour to the nose in the normal animal, is changed under strychnine to arrest in the inspiratory position, that is, this respiratory reflex is reversed in the same way as the reflex inhibition of skeletal muscle (Sherrington) or of the vasomotor reflex (Bayliss)<sup>1</sup> under strychnine. Seemann observed that this reversal is dependent on the passage of impulses from the lungs to the center, for when the vagus is cut the reflex resumes its normal expiratory character. He anaesthetised his rabbits with urethane and was thus able to inject very much larger quantities of strychnine (1 to 2 mg.) than could be used in decerebrated animals, in which I found

<sup>1</sup> But this vasomotor reversal is not confirmed by Langley (Journ. of Physiol. xlv, p. 239).

that 0.2 mg. was sufficient to cause violent convulsions, which returned on any attempt to arouse a reflex movement. I tested the nasal reflex in one anaesthetised rabbit under strychnine but did not obtain true reversal of the reflex. But the reversal under caffeine is similar to that described by Seemann, so that I lay no weight on my failure to obtain it in one experiment with strychnine. While I have been unable to induce reversal with strychnine in decerebrate animals, the inhibitory reflexes were changed to some extent by its injection in quantities of 0.1 mg. given once or twice in succession. The Hering-Kratschmer reflex was elicited by applying ammonia vapour to the nose, laryngeal inhibition was caused by ammonia in the larynx or by stimulation of the nerve trunk, and the expiratory vagus reflex by electrical stimulation of the nerve in the neck. In the last case both vagi were tied. In all cases the expiratory reflex could be elicited after 0.1 mg. strychnine or after several repetitions of this dose at intervals. But the inhibition was less complete and was of shorter duration. Thus a strength of current which before the injection caused complete arrest of the breathing lasting throughout the stimulation and often beyond it, was no longer sufficient afterwards, the pause being interrupted by slow, weak inspirations. After repeated injections, and sometimes even after a single dose, the complete arrest gave way to a slow, regular series of respirations. The respiratory reflex was unchanged in quality therefore but reduced in power. As in the case of caffeine, this change in the reflex was accompanied by increased activity of the center, as is shown by the acceleration and also by the augmented response to CO<sub>2</sub> inhalation; it may be due to the inhibitory reflex losing its control over the hypersensitive center rather than to any failure in the passage of impulses through the synapses on the afferent inhibitory path.

#### DISCUSSION

All the drugs examined—morphine, chloral, urethane, caffeine and strychnine—affect the frequency of the respiration more constantly than the depth; and as a general rule the change in the frequency is in the opposite direction to that in the depth. Thus

the three depressants, morphine, chloral and urethane, tend to slow and deepen the respiration, while strychnine and caffeine quicken it and render it shallower.<sup>2</sup> But considerable changes in the rate may occur under all these drugs without any significant change in the depth. The reaction to  $\text{CO}_2$  is altered in the same way as the respiration in air. For here again the chief alteration is shown in the frequency, while the depth is comparatively little changed. The view that the normal activity of the respiratory center is dependent on the  $\text{CO}_2$  hormone thus receives support from these experiments, in which drugs that reduce the normal rate of respiration also lessen the reaction to  $\text{CO}_2$ , and similarly drugs that increase the normal rate of respiration augment the reaction to  $\text{CO}_2$ .

As far as the frequency is concerned, in fact, these drugs might be said to affect the respiratory center simply by changing its sensitiveness to the specific hormone. But this does not hold for the depth of the breathing which is little affected by them directly. For the changes in the depth of the breathing under poisons arise not from a direct influence on the strength of the impulses emitted by the center, but from changes either in the  $\text{CO}_2$  production (from their general central action), or in the  $\text{CO}_2$  elimination (from their affecting the rate of the respiration). Drugs therefore affect only one of the relations between the respiratory center and its hormone, leaving the other unaltered.

As regards the nervous control of the respiration, those drugs that accelerate the breathing render the inhibitory reflexes less efficient, while the depressants facilitate inhibition. An analogy may be drawn between the effects of poisons and those of changing the number of afferent stimuli. For example the respiration of

<sup>2</sup> In clinical and experimental observations the breathing is often described as "slow and shallow," which can occur only if the  $\text{CO}_2$  tension is very low or if the center is affected in a way which has not occurred in my experiments. The reduction in the  $\text{CO}_2$  production is obviously the cause of the shallow respiration in general anaesthesia or collapse (Henderson), and it would be of interest to determine whether the inhalation of dilute  $\text{CO}_2$  would be useful in these conditions. Opium poisoning has long been treated by forcing the patient to walk about with the result that the  $\text{CO}_2$  production is increased and arouses the flagging center. The same result might be obtained more easily by  $\text{CO}_2$  inhalation.

profound morphine poisoning resembles in so many features that after section of the vagi, that it suggests that morphine may act by cutting off afferent stimuli from the center. But the poisons investigated have not proved to possess any very striking action on the passage of impulses to the center and such changes as are observed in the effects of reflex excitation may be regarded rather as the result of the changes in the center rather than as the essential cause of these changes.

The effects of drugs on the respiration therefore cannot be assigned wholly to either factor in the regulation. The relation of the hormone to the center is altered under drugs but only as regards the rate. Change in the nervous control occurs but also fails to account for all the features observed.

The essential character of the respiratory center is its power of rhythmic discharge under a constant stimulus from the presence of  $\text{CO}_2$ . The frequency of the rhythm and the strength of the impulses emitted, may be altered by variations in the  $\text{CO}_2$  tension and also by nervous influences, but the essential rhythmic power persists. Drugs quicken or slow the rhythm without necessarily altering the strength of the impulses sent out at each active phase; in other words, the rapidity of the anabolism in the center is changed by drugs but the strength of the catabolic discharge which occurs when the critical point is reached, is unchanged by them except in as far as they change the  $\text{CO}_2$  tension indirectly.

Thus the changes in the respiration under poisons cannot be attributed exclusively to altered susceptibility either to the hormone or to nervous impulses, but rather indicate a more fundamental action on the rhythmic process on which both of these influences act. This change in the rate of the anabolism affects the response to  $\text{CO}_2$ , which induces greater or less acceleration according as the anabolism is promoted or retarded. The afferent nervous impulses are also influenced, but the strength of the impulses emitted are changed only indirectly.

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# THE FACTORS DETERMINING TOLERANCE OF GLUCOSIDES OF THE DIGITALIS SERIES

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## I. INTRODUCTION

The experiments described in this paper were undertaken to determine, by what mechanism tolerance is established in the snake, and the rat, to glucosides of the digitalis series (for convenience these drugs will be referred to hereafter as "the cardiac glucosides").

The degree of susceptibility of any animal to a drug must depend upon some of the following factors:

1. The rate at which the drug enters the circulating fluids of the body, that is the rate at which the drug is absorbed from the gut or subcutaneous tissues.

2. The rate at which the drug is removed from the circulating fluids by excretion, by absorption by indifferent tissues, or by changes which render it inactive.

3. The minimal concentration that the drug must attain, in order to affect the tissues which are most susceptible to its action.

The object of the present paper is to determine upon which of these mechanisms tolerance of strophanthin depends, and also to learn by what mechanism the cumulative action of strophanthin is produced. With these objects in view experiments were made upon two cold blooded animals, the grass snake, an animal very tolerant of strophanthin, and the frog a susceptible animal, and secondly upon two mammals, the rat a tolerant animal, and the rabbit a susceptible animal.

The drug used in all experiments was "Wellcome" strophanthin, (B. & W.), a preparation from the seeds of *S. kombé*.

## II. EXPERIMENTS UPON COLD BLOODED ANIMALS

### A. *The frog*

The frog (*Rana temporaria*) is very susceptible to strophanthin, the minimal lethal subcutaneous dose of "Wellcome" strophanthin was just under 1 mg. per kilo body weight.

1. *Absorption of cardiac glucosides from subcutaneous tissues.* Cardiac glucosides are usually standardised by determining the minimal lethal subcutaneous dose in frogs. Focke (10) however pointed out that different glucosides could not be accurately compared by this method, because the insoluble glucosides were absorbed more slowly than the soluble, and therefore larger doses of the former were required to kill. This difference is seen clearly when digitoxin and strophanthin are compared; the minimal doses required to kill a 20 gram frog in 12 hours are respectively 0.12 mg. and 0.02 mg. a ratio of 6 to 1, but the minimal concentrations required to produce systolic arrest in an isolated frog's heart are respectively 0.0012 mg. per cubic centimeter and 0.0005 mg. per cubic centimeter, a ratio of only 2.5 to 1 (1). The rate of absorption from the subcutaneous tissues therefore plays some part in determining the action of the drug.

Strophanthin when injected subcutaneously was found to enter the blood stream in a considerable concentration.

*Experiment 1.* Strophanthin 0.2 mg. injected subcutaneously into each of six frogs, the frogs all died in thirty minutes, they were then

skinned and the bodies washed well with water to remove any of the drug left in the subcutaneous tissues. The hearts were exposed and the blood collected from the auricles, and made up to 2 cc. with Ringer; the fluid was tested upon an isolated frog's heart, which was killed in systole in ten minutes; this corresponds to at least 0.01 mg. strophanthin in the blood.

2. *Absorption of digitalis by the frog's heart.* When a cardiac glucoside is injected into a frog, the frog continues to hop about apparently unaffected for some time after its heart has been arrested in systole; this shows that the cardiac glucosides have an extremely specific action upon the heart. Straub (23) and Cloetta and Fischer (4) agree however that it is impossible to recover cardiac glucosides from the hearts of frogs killed by these drugs, and Straub further showed that isolated frogs' hearts exposed to ouabain (g. strophanthin) at the most only absorbed a minute trace of the drug; this result was confirmed by the writer with strophanthin kombé (1); for, when this drug was circulated in isolated frogs' hearts, each heart absorbed only 0.00008 mg. or less of the drug. Gruenwald (12) on the contrary concluded that isolated frogs' hearts absorbed, or destroyed, large quantities of digitalin, but this drug is almost insoluble and he did not exclude the possibility that the loss of strength, observed after perfusion, may be due to precipitation of the drug by some constituent of the perfusion fluid; the writer (1) found that dilute solutions of digitoxin in Ringer rapidly lost in strength on standing, and therefore discarded this drug in favour of the freely soluble strophanthin, solutions of which in Ringer maintain their strength unimpaired for twenty-four hours.

The power of the frog's heart to absorb or destroy strophanthin was also tested by incubating the drug with emulsions of heart tissue, and, after centrifugalising the mixture, testing the supernatant fluid upon isolated frogs' hearts; it was found that, if no drug was added, such fluids had no injurious effect upon the isolated heart, so long as emulsions of frog's heart or skeletal muscle were used; but with other tissues such fluids rapidly killed isolated hearts, and in such cases chemical extraction was necessary.

The frogs' hearts used as tests in these experiments were isolated in the manner described in a previous paper (1). A cannula as shown in the figure (fig. 1) was fixed in the inferior vena cava, another cannula in the left aorta, the remaining vessels of the heart were ligatured, and the heart excised; the apparatus was fixed upon cork by means of pins, and movements were recorded by fine clips fixed to the auricles and ventricle, and connected

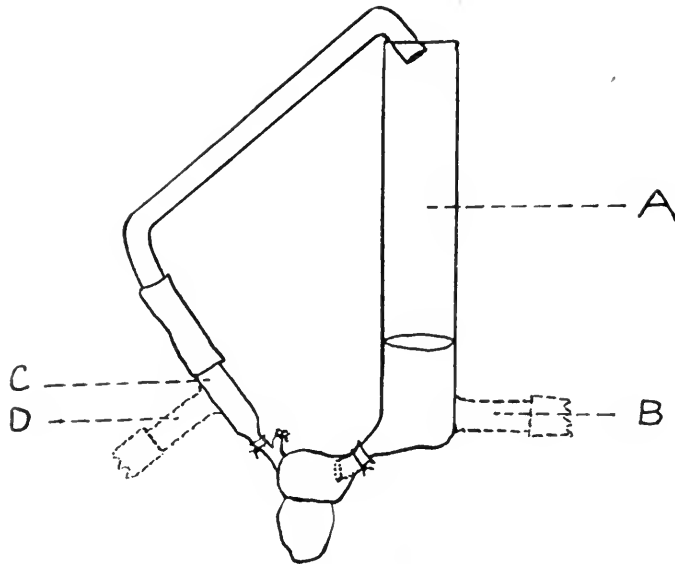


FIG. 1. APPARATUS FOR CIRCULATING A SMALL QUANTITY OF FLUID IN AN ISOLATED FROG'S HEART

A, venous cannula; C, aortic cannula. The dotted lines show T-tubes by means of which the isolated heart can be perfused with a large volume of fluid. B, inflow (from Marriotte's bottle); D, outflow.

with light straw levers. By means of this apparatus 1 or 2 cc. of fluid could be circulated in the heart for several hours. Strophanthin in a concentration of 0.001 mg. per cubic centimeter just produces systolic arrest of the frog's heart in one hour; therefore the strophanthin present in fluids was estimated by diluting the fluid until it just failed to produce systolic arrest within an hour.

In all experiments Ringer of the following composition was

used NaCl 0.7 per cent, KCl 0.014 per cent,  $\text{CaCl}_2$  0.012 per cent,  $\text{NaHCO}_3$  0.02 per cent.

*Experiment 2.* The hearts of six frogs were made into an emulsion with 2 cc. of Ringer, strophanthin was added to make a concentration of 0.001 mg. per cubic centimeter, and the mixture was thoroughly shaken and incubated at 20°C. for thirty minutes; and then centrifugalised; the supernatant fluid was tested by circulating in an isolated frog's heart which died in systole within an hour; this corresponds to a concentration of strophanthin of 0.001 mg. per cubic centimeter, and therefore no loss of strophanthin had occurred.

*Experiment 3.* The hearts of six frogs were made into an emulsion with 2 cc. of Ringer and strophanthin was added to make a concentration of 0.001 mg. per cubic centimeter. The mixture was incubated at 22°C. for three hours and was then centrifugalised, and the supernatant fluid tested upon an isolated frog's heart; systolic arrest was produced within an hour, and therefore no loss of strophanthin had occurred.

These results confirm the previous conclusion that the frog's heart does not absorb or destroy strophanthin.

3. *Power of indifferent tissues of the frog to absorb or destroy strophanthin.*—(a) Skeletal muscle. Experiments with emulsions of skeletal muscle and strophanthin did not indicate any absorptions of the glucoside by the muscle cells. In other experiments the frogs' legs were perfused with solutions of strophanthin.

*Experiment 4.* A frog was pithed, the abdomen opened and cannulae inserted into the abdominal aorta, and the anterior abdominal vein. The rectum and bladder were ligatured, and the abdominal contents removed; ligatures were then tied round the abdominal walls, the aorta and abdominal vein being excluded. The frog was fixed on a piece of cork, the legs hanging down vertically; the preparation was perfused through the aorta with Ringer at a pressure of 10 cm. of fluid, and the outflow from the vein collected. The preparation was perfused until a regular flow of clear fluid came from the vein and then (1) 5 cc. fluid containing 0.012 mg. strophanthin were perfused, and 2.5 cc. collected from the outflow; (2) 10 cc. more fluid were perfused and 3.5 cc. collected from the outflow. The frogs' legs were found to be very oedematous so cuts were made into the skin and muscles and the preparation allowed to drain; 5 cc. of fluid were collected in this manner. A total of 11 cc.

fluid was thus collected, this killed an isolated frog's heart in systole in an hour, corresponding to a concentration of 0.001 mg. strophanthin per cubic centimeter, or a total quantity of 0.011 mg. strophanthin. There is therefore no evidence that the legs absorbed any of the drug.

In another experiment 0.005 mg. strophanthin in 10 cc. Ringer was perfused through frogs' legs prepared in a similar manner except that, to avoid oedema, the renal portal veins were not ligatured and slits were made in the skin of the legs. In this case 10 cc. fluid containing 0.005 mg. strophanthin were passed in and 6 cc. containing 0.003 mg. strophanthin were collected; therefore the tissues had not specifically absorbed any strophanthin.

The tissues of the leg have therefore no power to retain strophanthin perfused through them.

(b) Absorption or destruction by liver. Emulsions of the liver were used, and it was found necessary to isolate the strophanthin by chemical extraction: the following method was employed.

The liver was taken from a freshly killed animal, washed with Ringer, and ground up in a mortar with sand, and filtered through muslin. Two equal portions of emulsion were taken and one portion killed either by heating to 60°C. for thirty minutes, or by the addition of alcohol in excess; this portion served as a control against losses due to chemical manipulation. A few drops of toluol and an equal quantity of strophanthin were added to each portion, and both were incubated together.

Extraction was performed by adding to the emulsion ten times its volume of absolute alcohol, warming for thirty minutes on a water bath and filtering; the residue was re-extracted with alcohol and the mixed filtrates evaporated slowly to the consistency of a paste. Excess of absolute alcohol was next added, the mixture was filtered, the residue re-extracted, and filtered, and the filtrates slowly evaporated to dryness on a water bath. The residue was washed with petrol ether, and then dissolved in a few cubic centimeters of 0.1 per cent HCl; the fluid was then neutralised, filtered, and made up to a known volume with Ringer. The quantity of strophanthin present in the extract was determined by circulating it in frogs' hearts according to the method already described.

In control experiments it was found that it was rarely possible to recover the whole of the strophanthin, owing to the unavoidable loss due to chemical manipulation, moreover the method of estimation, although extremely delicate, gives results with a possible error of 30 per cent. Control experiments were therefore always performed, and the loss due to chemical manipulation thus allowed for: also the smallest quantities of drug were always employed that could be recovered with certainty from the control, so that if any destruction of the drug occurred there should be a large percentage difference between the drug recovered in the experiment and in the control. In a similar series of experiments conducted with atropine (3) it was found that very definite results were obtained, as emulsions of certain tissues completely destroyed the drug, whilst other tissues destroyed none at all.

Cloetta and Fischer (4) estimated digalen in extracts from tissues by means of a colour reaction, but this method does not demonstrate quantities below 0.04 mg. and prolonged chemical treatment is required to remove all pigments from the extracts so that I consider it much inferior to the method described in this paper. Hatcher (14) demonstrated ouabain in animal tissues by injecting watery extracts intravenously into cats, this method demonstrates 0.01 mg.; it is more accurate than the isolated heart method, but it has the disadvantage of being expensive, and it is not very delicate.

*Experiment 5.* The livers of six frogs were made into an emulsion and Ringer added to make 6 cc., the fluid was divided into two portions A and B, and portion B was heated to 60°C. for thirty minutes. One drop of toluol, and 0.05 mg. strophanthin, were added to each portion, and the mixture incubated at 22°C. for sixteen hours; the emulsions were then extracted in the manner described, and the extracts tested upon isolated frogs' hearts. Both of the extracts when diluted to 100 cc. produced semi-systolic arrest of the hearts in two hours, when diluted to 50 cc. they both produced systolic arrest within one hour (fig. 2). A concentration of 0.0005 mg. strophanthin per cubic centimeter produces semi-systolic arrest of the heart in two hours, and a concentration of 0.001 mg. per cubic centimeter produces systolic arrest in one hour.

The extracts therefore both contained about 0.05 mg. strophanthin, and no destruction of strophanthin had occurred.

(c) Absorption or destruction in frog's blood. The presence of frog's blood was found distinctly to diminish the action of strophanthin on the frog's heart.

*Experiment 6.* One cubic centimeter of defibrinated frog's blood was mixed with 0.0012 mg. strophanthin dissolved in 0.1 cc. Ringer, and circulated in an isolated frog's heart. The heart was unaffected after two hours.

*Experiment 7.* One cubic centimeter defibrinated frog's blood was mixed with 0.0014 mg. strophanthin and circulated in an isolated frog's heart. The heart was unaffected after two hours.

*Experiment 8.* One cubic centimeter frog's blood was mixed with 0.002 mg. strophanthin, and incubated at 20°C. for thirty minutes, and then put into an isolated frog's heart. The heart was unaffected after two hours.

As already mentioned 0.0005 mg. strophanthin in 1 cc. Ringer produced arrest of an isolated frog's heart in two hours. Frog's serum does not alter the minimal lethal concentration of strophanthin.

*Experiment 9.* One cubic centimeter frog's serum mixed with 0.0003 mg. strophanthin, incubated one hour at 20°C., and then circulated in an isolated frog's heart. The heart was unaffected after two hours.

*Experiment 10.* One cubic centimeter frog's serum mixed with 0.001 mg. strophanthin, incubated one hour at 20°C. and then circulated in an isolated frog's heart. Systolic arrest was produced within an hour.

Werschinin (25) states that the presence of serum intensifies the action of strophanthin, but he estimated the action of strophanthin by the minimal concentration that produced systolic arrest in Williams' apparatus. As I have suggested in a previous paper (1) his results were probably due to serum improving the condition of the hearts, and causing them to react more vigorously to the drug. With my apparatus the heart works under a much lower pressure than in the Williams' apparatus, and much smaller quantities of fluid are used, consequently the heart



remains in a much better condition, and serum has little influence on its reaction to strophanthin.

The difference in the results obtained with defibrinated blood and serum would be explained if the red blood corpuscles could absorb strophanthin, and experiments were made to determine this point.

*Experiment 11.* One cubic centimeter defibrinated blood was mixed with 0.0014 mg. strophanthin, incubated for one hour, and then centrifuged, and the supernatant serum tested on an isolated heart. The heart was unaffected after two hours.

*Experiment 12.* One cubic centimeter defibrinated blood was mixed with 0.0025 mg. strophanthin, and treated in the same manner as in the last experiment. Systolic arrest of the isolated heart was rapidly produced.

These experiments, and other similar ones, showed that the red blood corpuscles absorbed a certain small quantity of strophanthin, but that this power was very limited. Attempts were made to recover strophanthin from red blood corpuscles treated in the manner described above, by laking the corpuscles either with water or by freezing, but no strophanthin could be recovered. Other experiments were made with washed red blood corpuscles of the frog suspended in Ringer to ascertain if these could absorb strophanthin, but the corpuscles under these conditions appeared to have no power to absorb the drug; their power to absorb strophanthin appears therefore to depend upon the presence of serum.

The experiments upon the frog show therefore that none of the tissues tested have any power to absorb or destroy strophanthin, with the single exception of the red blood corpuscles, and these in the presence of serum can remove small quantities of the drug from solution.

3. *The minimal concentrations of strophanthin required to affect the different tissues of the frog.* As already mentioned strophanthin has a very markedly selective action on the frog's heart. Fraser and Mackenzie (10) showed that in the intact frog the nervous system, and the skeletal muscle, were not affected until

after systolic arrest of the heart had been produced. The writer compared the action of strophanthin upon the isolated heart, arterioles, stomach muscle, and skeletal muscle. The isolated heart was killed by a concentration of strophanthin of 0.0005 mg. per cubic centimeter. When frogs' legs were perfused with solutions of strophanthin and the rate of flow recorded, 0.1 mg. per cubic centimeter was required to produce vaso-constriction; with digitoxin however a concentration of 0.02 mg. per cubic centimeter produced well marked vaso-constriction. With isolated rings of stomach muscle a concentration of 0.1 mg. strophanthin just produced a definite constriction. With isolated skeletal muscle a concentration of 1 mg. per cubic centimeter killed the muscle in a relaxed condition but smaller concentrations had no apparent effect (2). The heart therefore is very much more susceptible to strophanthin than any other tissue.

*B. The grass snake (Tropidonotus natrix)*

1. *The cause of tolerance to strophanthin in the grass snake.* The common grass snake was found to be very tolerant of strophanthin, the minimal lethal dose of the drug, injected hypodermically, being 30 mg. per kilo body weight: this dose killed the snake and produced systolic arrest of the heart, while in the frog this effect followed the hypodermic injection of 1 mg. per kilo body weight. The tolerance of the snake was found to be completely explained by the immunity of the heart tissue, for the isolated snake's heart was unaffected by a concentration of 0.5 mg. strophanthin per cubic centimeter of fluid, while 1 mg. per cubic centimeter produced a slight systolic effect, but a concentration of 2 mg. per cubic centimeter was required to produce systolic arrest within two hours, an effect produced on the isolated frog's heart by 0.0005 mg. per cubic centimeter. The intact snake is therefore only thirty times more tolerant than the frog to strophanthin injected hypodermically, but the isolated snake's heart is more than one thousand times more tolerant than the isolated frog's heart. This immunity of the snake's heart to strophanthin is not due to any action of Ringer's fluid on the heart, for similar

results were obtained when the snake's heart was perfused with defibrinated snake's blood containing strophanthin.

The toad is the only other cold blooded vertebrate known to be tolerant to cardiac glucosides, but in this animal a digitalis like body is present in the blood stream; Heuser (16) has shown that the toad is unaffected by a hypodermic injection of strophanthin sufficient to kill 100 frogs, he also showed that after injection of strophanthin there was a definite increase in the cardiac glucosides present in the blood stream some hours after the injection, this indicates that in the toad the strophanthin injected reaches the heart, and that immunity is due to insusceptibility of the heart tissue, and not to non-absorption, or to rapid excretion of the drug.

In invertebrates Straub (quoted by Evans (6)) has shown that the heart of *Aplysia* is not affected by strophanthin, and Evans (6) has shown the same in the isolated heart of *Helix pomatia*. Immunity of the heart tissue to strophanthin appears therefore to be a widely spread phenomenon.

Experiments were made with snake's blood to determine whether any digitalis like body was present in it. Snake's blood, serum and plasma were all found to be equally toxic to intact frogs, 0.5 cc. killing an intact frog, when injected hypodermically, in about twenty-four hours; but the frogs were killed with their hearts in diastole, and moreover snake's blood or plasma, diluted ten times with Ringer, and circulated in isolated frog's hearts, produced no ill effects upon them even after several hours. There is therefore no substance resembling digitalis present in the snake's blood, and the explanation of the toad's tolerance of cardiac glucosides does not apply in the case of the snake.

2. *Distribution of strophanthin in the snake's body.* When strophanthin was injected into snakes large amounts were found in the blood, but not in other organs.

*Experiment 13.* A grass snake (weight 26 grams) was injected with 1.3 mg. strophanthin (= 50 mg. per kilo body weight), and died in one hour. The blood from the heart was diluted four hundred times and 2 cc. circulated in an isolated frog's heart, which it killed in systole;

this corresponds to a concentration of strophanthin in the snake's blood of more than 0.2 mg. per cubic centimeter.

*Experiment 14.* A grass snake (weight 24 grams), was injected with 0.24 mg. strophanthin hypodermically (= 10 mg. per kilo body weight), the drug had no apparent injurious effect upon the snake. After sixteen hours the snake was killed, and 0.7 cc. blood was collected, and diluted to 7 cc. with Ringer; 2 cc. of this fluid was circulated in an isolated frog's heart, and produced a definite systolic effect after two hours, which corresponds to a strength of strophanthin of about 0.005 mg. per cubic centimeter of blood. The heart, liver, and gut contents, were all extracted and the extracts tested for strophanthin upon isolated frogs' hearts; in no case was any strophanthin detected.

These experiments show that when strophanthin is injected in the snake it enters the blood stream, and remains in it for a long time, but no organ appears to have the power to absorb strophanthin.

3. *Destruction of strophanthin by the snake's heart.* Experiments were made with the isolated snake's heart to see if it could destroy strophanthin.

*Experiment 15.* A solution of Ringer containing about 0.25 mg. strophanthin per cubic centimeter was prepared, one portion was kept as a control, and 2 cc. were circulated in a snake's heart; this concentration of the drug did not injure the heart (fig. 2) and after four hours the fluid was removed, and the amount of strophanthin present determined, by finding the minimal quantity of fluid that, when injected hypodermically, would kill a 20 gram frog, by producing systolic arrest of the heart. With the control solution 0.9 cc. killed a 20 gram frog with its heart in systole in twelve hours, while 0.7 cc. did not kill a 20 gram frog in 12 hours; 1.2 cc. of the fluid which had circulated in the snake's heart, killed a 20 gram frog, with systolic arrest of the heart, in twelve hours. The minimal dose of strophanthin that produced systolic arrest of the heart of a 20 gram frog within twelve hours was 0.02 mg. The stock solution therefore contained 0.22 mg. per cubic centimeter and, after circulating in the heart, it contained 0.17 mg. per cubic centimeter or more.

*Experiment 16.* Two cubic centimeters of a fluid, containing about 0.2 mg. strophanthin per cubic centimeter, were circulated in an isolated snake's heart for three hours. The stock solution, tested as in experi-

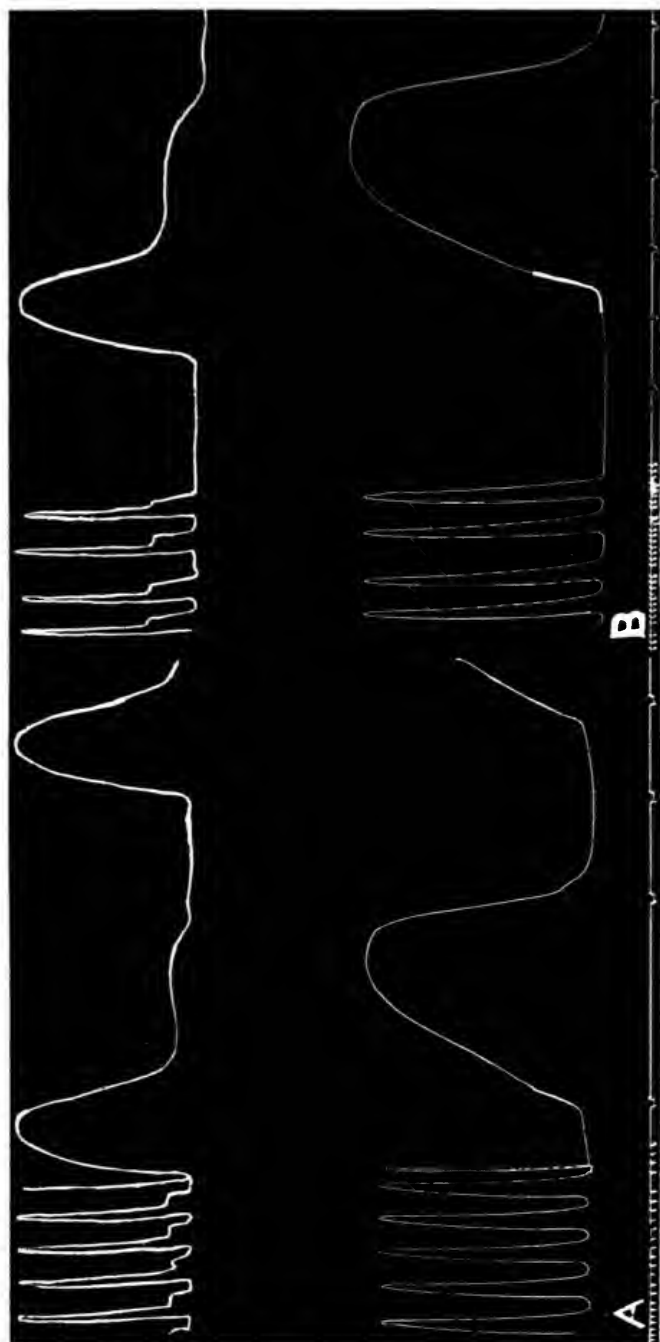


FIG. 2. ACTION OF STROPHANTOIN UPON ISOLATED SNAKE'S HEART

Two cubic centimeters of ringer circulated through heart. A, one hour after isolation; immediately after .4 0.5 mg. strophantoin added to fluid. B, four hours later. Upper tracing, auricular movements; lower tracing, ventricular movements. Upstroke systole. Time in seconds. Read from left to right.

ment 15, was found to contain between 0.2 and 0.24 mg. strophanthin per cubic centimeter, and the fluid which had circulated three hours contained between 0.17 and 0.2 mg. per cubic centimeter.

Both of these experiments show a possible loss of about 20 per cent of the strophanthin present, but the method of estimation is only accurate within 10 per cent, and simple diffusion of the drug into the heart tissue would account for a loss of about 10 per cent, therefore the snake's heart does not destroy any large percentage of strophanthin circulated in it, but it may destroy or absorb up to 20 per cent of the drug, this possible loss may however be accounted for by diffusion into the heart muscle, and experimental error.

4. *Absorption of strophanthin by the isolated snake's heart.* Experiments were performed to determine whether the snake's heart had any power specifically to absorb strophanthin. The previous experiments rendered this improbable, but further evidence was sought for by extracting hearts, that had been perfused with solutions of strophanthin.

*Experiment 17.* The hearts of two snakes were isolated and perfused for several hours with strophanthin in concentrations of from 0.2 to 2 mg. per cubic centimeter; both hearts were then washed out thoroughly with Ringer, to remove any of the drug present in the coronary vessels; the hearts were then extracted (as described p. 404) and the extract tested on isolated frogs' hearts. The extract when diluted to 25 cc. produced systolic arrest of the isolated frog's in an hour, but when further diluted it failed to do so; this action corresponds to that produced by a concentration of strophanthin of 0.001 mg. per cubic centimeter. The extract therefore contains about 0.025 mg. strophanthin. Since the two hearts weighed 0.5 gram, the concentration of strophanthin was about 0.05 mg. per gram of muscle, which is much lower than the concentration in the perfusion fluid.

*Experiment 18.* Two cubic centimeters of fluid, containing 0.5 mg. strophanthin per cubic centimeter were circulated through an isolated snake's heart for three hours. At the end of this time 0.98 cc. of fluid was removed, the snake's heart was cut into strips and dried by pressing gently between filter papers and then weighed; its weight was 0.211 gram. The fluid and the heart were extracted separately, and great

care was taken to treat both in an exactly similar manner, so that any loss of strophanthin due to chemical manipulation should be equal in the two cases. The extracts were tested for strophanthin both on intact frogs and on isolated frogs' hearts.

(a) The extract of the perfusion fluid. One-twentieth of this killed a 20 gram frog in three hours, one-thirty-third did not kill a 20 gram frog in twelve hours; the total extract contains therefore about 0.4 mg. strophanthin. When tested on the isolated heart the extract, diluted with 600 cc. of fluid produced semi-systolic arrest in two hours, while diluted to 300 cc. it produced systolic arrest in one hour; this corresponds to a total of 0.3 mg. strophanthin.

(b) The extract of the heart. One-seventh of this had no effect upon a 20 gram frog; but when the extract was diluted to 10 cc. 2 cc. just produced systolic arrest of an isolated frog's heart within one hour; this corresponds to a total quantity of 0.01 mg. strophanthin in the heart extract.

The amount of strophanthin recovered therefore corresponded to a concentration in the fluid of 0.3 to 0.4 mg. per cubic centimeter, and to a concentration in the heart muscle of 0.05 mg. per gram.

*Experiment 19.* Two cubic centimeters of a solution of 0.5 mg. strophanthin per cubic centimeter were perfused through an isolated snake's heart for two hours. The fluid and the heart were treated in exactly the same manner as in the last experiment. It was found that the strophanthin recovered from the fluid corresponded to a concentration of 0.4 mg. strophanthin per cubic centimeter, and that recovered from the heart to a concentration of 0.01 mg. per gram of heart muscle.

These last two experiments show that the concentration of strophanthin in the heart muscle is much less than the concentration in the fluid perfusing through the heart, the heart muscle does not therefore specifically absorb strophanthin in a form that can be recovered by extraction.

Previous experiments (experiments 15 and 16) showed that the heart did not remove more than 20 per cent of the total quantity of strophanthin that had circulated in it for many hours. The snake's heart therefore certainly does not specifically absorb strophanthin, in any form that can be recovered, and although the heart may destroy small quantities of strophanthin, the evidence for this is inconclusive; this possible destruction is more-

over quite insufficient to account for the immunity displayed by the heart to strophanthin; in this connection it is noteworthy that the concentration of the strophanthin in the heart muscle in experiments 18 and 19 was high enough to kill rapidly the heart of any other animal that I have investigated if perfused through it; the snake's heart offers therefore a very striking example of tissue immunity to strophanthin.

The tolerance of strophanthin shown by the snake depends therefore entirely upon tissue immunity, the drug when injected enters the blood stream and is only slowly removed from it, probably by excretion, and the tissues do not appear to have any power specifically to absorb the drug.

### III. EXPERIMENTS UPON MAMMALS

Hatcher and Bailey (15) investigated the fate of ouabain when administered to mammals. They showed that the absorption of the drug from the gut was extremely irregular, and that, in the dog, up to 20 per cent of the amount administered might be destroyed in the gut. Deucher (5) and Holste (17) both showed that intestinal ferments might destroy certain cardiac glucosides *in vitro*. Hatcher and Bailey (15) also showed that the amount of the drug absorbed from the gut varied greatly, according to the manner in which it was administered; the normal minimal lethal dose of ouabain by mouth in a cat or dog was found to be about ten times the minimal lethal intravenous dose, and in animals in poor condition the minimal lethal dose by mouth might even be higher, but when ouabain was given to a fasting cat by mouth in a concentrated alcoholic solution, death might be produced by a dose only twice the minimal lethal intravenous dose. These results show that the administration of cardiac glucosides by mouth must always produce very uncertain results, and that the normal difference, between the minimal lethal intravenous dose of strophanthin, and the lethal dose by mouth, is accounted for by the slowness with which the drug is absorbed, and its destruction by the intestinal ferments. Fraenkel (9) showed that different cardiac glucosides were ab-



sorbed at different rates from the subcutaneous tissues in mammals, but that slowness of absorption would not account for the cumulative effects observed. Hatcher (14) showed that the rat was very tolerant of ouabain, but that nearly all of a dose of ouabain injected hypodermically was excreted by the kidneys and gut within twenty-four hours; therefore the tolerance is not due to difficulty of absorption from the subcutaneous tissues. Hatcher also showed that when ouabain was given subcutaneously to a rat about 10 per cent of the drug was excreted by the kidneys within six hours, and that 66 per cent was excreted into the gut within twenty-four hours. In one experiment he recovered 87 per cent of the dose injected from the excretions within twenty-four hours; he concluded from these experiments that it was improbable that any of the body tissues could absorb or destroy ouabain.

Cloetta and Fischer (4) injected digalen subcutaneously and showed that in the rabbit, the drug rapidly appeared in the urine, and that one hour after injection the drug was still present in the blood. These results suggest that the drug is not absorbed by the tissues, but in one experiment on rats they recovered from the heart muscle no less than 12 per cent of the amount injected; from this they conclude that the heart had a specific power to absorb digalen. Hatcher repeated this experiment with ouabain but was unable to recover any of the drug from the heart muscle. Hatcher used a more powerful drug than Cloetta and Fischer and his method of estimating the drug was more reliable, and more delicate, it seems probable therefore that Cloetta and Fischer's result was due to experimental error. Schliomensum (22) showed that alcoholic extracts of heart muscle possessed, the power either to precipitate or to destroy digitoxin, but since this drug is very insoluble and is fairly readily broken down, this observation does not prove any special affinity between heart muscle and digitoxin. Roger (21) and Hatcher and Bailey (15) both showed that the mammalian liver did not absorb any strophanthin when the drug was injected into the portal vein. It appears therefore that the mammalian tissues neither absorb nor destroy strophanthin, but the evidence is not

conclusive, and the question is of considerable importance; for the cardiac glucosides have a markedly cumulative action when the drug is given by mouth, or subcutaneously (Fraenkel (9)); and this action would be readily explained if it could be shown that the heart tissue could absorb strophanthin. Experiments therefore were performed with mammalian tissues to determine whether they could either absorb or destroy strophanthin; rabbits and rats were used, the former being susceptible to, and the latter tolerant of, strophanthin.

#### A. *The rabbit*

The rabbit is susceptible to cardiac glucosides, the minimal lethal intravenous dose of strophanthin being 0.5 mg. per kilo body weight, and of ouabain 0.3 mg. Gley (11).

1. *Absorption of strophanthin by the rabbit's heart.* Fahrenkamp (7) found that ouabain, at a concentration of 0.000025 mg. per cubic centimeter, killed an isolated rabbits heart in one hour. Gunn (13) using a dried extract of strophanthin, which was one-third as toxic to the intact rabbit as ouabain, found that a concentration of 0.0001 mg. per cubic centimeter killed an isolated rabbit's heart in forty minutes. Using "Wellcome" strophanthin

TABLE I

NUMBER OF EXPERIMENT	APPARATUS USED	CONCENTRATION OF STROPHANTHIN IN MILLIGRAMS PER CUBIC CENTIMETER	QUANTITY (IN MILLIGRAMS) OF STROPHANTHIN INJECTED INTO SIDE TUBE	EFFECT
20	Langendorff's	0.00005		Systolic arrest in 40 minutes
21	Langendorff's	0.00004		Diastolic arrest in 75 minutes
22	Brodie's	0.00004		Systolic arrest in 25 minutes
23	Langendorff's	0.0002		No effect in 120 minutes
24	Brodie's		0.1	Systolic arrest in 30 minutes
25	Brodie's		0.05	Systolic arrest in 20 minutes
26	Brodie's		0.025	No effect in 120 minutes
27	Brodie's		0.02	No effect in 120 minutes

thin, one-half as toxic as ouabain to the intact frog, the writer obtained the results given in table I with isolated rabbits' hearts. Some of the experiments were performed with Brodie's apparatus, and others with a modification of Langendorff's apparatus; in some experiments dilute solutions of strophanthin were perfused for long periods, in other experiments a known quantity of strophanthin was injected into the perfusion fluid, by a side tube close to the heart.

In all experiments the rate of flow maintained was less than 5 cc. per minute, and strophanthin at a concentration of 0.00004 mg. per cubic centimeter killed the heart in twenty-five minutes, that is after less than 0.005 mg. of strophanthin had passed through the heart; but 0.025 mg. strophanthin when injected in concentrated solution did not kill the heart, this indicates that the heart does not retain strophanthin, when it is perfused through it, and that the drug requires to be in contact with the heart a considerable time in order to produce any change in the muscle. In many of the experiments in which concentrated solutions of strophanthin were injected, the fluid, after passing through the rabbit's heart was tested upon isolated frogs' hearts, in no case was there any evidence that the heart absorbed strophanthin; for instance, in experiment (26) 0.02 mg. strophanthin was injected into the perfusion fluid, and 0.015 mg. was demonstrated in the next 10 cc. of fluid, that passed through the heart.

2. *Absorption of strophanthin by the rabbit's liver.* In these experiments the isolated liver was perfused through the portal vein with Ringer containing strophanthin; the liver was kept in a hot air chamber at 37°C., and oxygenated Ringer was supplied from a reservoir, at a pressure of about 10 cm. of water, and after perfusion the fluid was returned to the reservoir by a small pump, in this manner 500 cc. of fluid could be circulated through the liver for many hours.

*Experiment 28.* The liver was perfused for three hours with 350 cc. Ringer containing 2 mg. strophanthin. At the commencement of the experiment 1 cc. of fluid was removed and tested upon isolated frogs' hearts; when diluted to 5 cc., the fluid produced systolic arrest within

an hour, but when diluted to 7 cc. it did not do so. After circulating through the liver three hours the fluid was again tested in the same way and the concentration of strophanthin found to be unaltered. The fluid and the liver were both extracted and the extracts tested for strophanthin; slightly over 0.5 mg. of strophanthin was recovered from the perfusion fluid but no strophanthin could be demonstrated in the extract from the liver, although by the method adopted the presence of 0.04 mg. of strophanthin in the extract would have been demonstrated.

Several other similar experiments were performed but in no case was there any evidence of the liver absorbing or destroying strophanthin.

### 3. *Destruction of strophanthin by the rabbit's liver.*

*Experiment 29.* An emulsion was made of fresh rabbit's liver by pounding it with sand in a mortar, and filtering through muslin; the emulsion was divided into two equal portions of 13 cc. each, and to each was added a few drops of toluol, and 1.0 mg. strophanthin.

Mixture A. Incubated at 37°C. for one hour.

Mixture B. Absolute alcohol 130 cc. added and then incubated at 37°C. for one hour.

Both portions were then extracted and the extracts tested upon isolated frogs' hearts. A quantity corresponding to 1 mg. was recovered from extract (A), and a quantity corresponding to 0.5 mg. from extract (B).

The method employed for estimating the drug was suitable for detecting minute quantities, rather than for estimating accurately the total quantity recovered, and the difference in the two quantities recovered is probably due to experimental error, there is however no indication that the emulsion can destroy strophanthin.

4. *Absorption of strophanthin by rabbit's blood.* Defibrinated blood was mixed with strophanthin, incubated, then centrifugalised, and the plasma tested upon isolated frogs' hearts, but there was no evidence that the mammalian blood corpuscles could absorb strophanthin in the manner that the frog's blood corpuscles did.

### B. The rat

The rat is very tolerant of strophanthin, the minimal lethal dose in the adult white rat was between 30 and 60 mg. of "Wellcome" strophanthin per kilo body weight. Gunn (13) using an extract of strophanthin found the minimal lethal subcutaneous dose to be 30 mg. per kilo, Hatcher (14) found that over 100 mg. of ouabain per kilo were required to kill a full grown rat.

The reason for this tolerance was shown by Gunn to be insusceptibility of the heart tissue, for a concentration of 0.002 mg. strophanthin per cubic centimeter of perfusion fluid was required to produce systole within one hour in the isolated rat's heart, and this is thirty times the concentration required to produce the same effect in the rabbit's heart. The writer found that the isolated rat's heart, when kept at a temperature of 25°C., was just killed in systole by a concentration of 0.005 mg. strophanthin per cubic centimeter the heart was also killed by 0.1 mg. strophanthin injected into a side tube close to the heart and thus passed through the heart in a concentrated solution.

1. *Absorption of strophanthin by the rat's heart.* No strophanthin was recovered on extraction of hearts perfused with strong solutions of the drug.

*Experiment 30.* A rat's heart was perfused with 0.01 mg. strophanthin per cubic centimeter perfusion fluid, at a temperature of 25°C., the heart was killed in systole in four hours. The heart was washed out with Ringer and then extracted, and the extract tested upon an isolated frog's heart, but no strophanthin could be demonstrated. Other similar experiments also gave negative results.

No loss of strophanthin could be demonstrated, when solutions of strophanthin were perfused through rats' hearts.

*Experiment 31.* A rat's heart was perfused with Brodie's apparatus, and 0.005 mg. of strophanthin was injected into a side tube. The first 5 cc. of fluid passing through the heart after injection of the drug were collected, and tested upon an isolated frog's heart; a quantity of strophanthin was demonstrated corresponding to 0.0037 mg. In another experiment 0.01 mg. strophanthin was perfused and 0.007 mg. was demonstrated in the fluid leaving the heart. The rat's heart does not

therefore absorb more than a trace of strophanthin and it is doubtful if it absorbs any of the drug.

5. *Absorption of strophanthin by the rat's liver.*

*Experiment 32.* A rat's liver was perfused in the manner described in experiment 28. The perfusion fluid contained 1 mg. strophanthin, and portions of it were tested upon isolated frogs' hearts both at the commencement of the perfusion, and at the end of three hours' perfusion, equal quantities of strophanthin were demonstrated in both cases, corresponding to a total of 1 mg. in the fluid. After perfusion for three hours the fluid and the liver were extracted separately and 0.5 mg. of strophanthin was recovered from the fluid, but none from the liver.

6. *Destruction of strophanthin by the rat's liver.*

*Experiment 33.* An emulsion of rat's liver was divided into two portions of 3 cc. each, to each portion was added a drop of toluol, and 0.05 mg. strophanthin, one portion was killed at once by the addition of excess alcohol, and both were incubated for five hours at 37°C. The emulsions were then extracted and the extracts tested upon isolated frogs' hearts; 0.01 mg. strophanthin was demonstrated in each portion.

Other experiments gave similar results, in no case was there any evidence of the liver destroying strophanthin.

7. *Absorption of strophanthin by the rat's blood corpuscles.* Experiments were made with rat's blood similar to those described in the case of the frog, the rat's blood possessed no power to absorb, or to destroy strophanthin, even after prolonged incubation.

There is therefore no evidence that any of the mammalian tissues investigated possess any power to absorb or to destroy strophanthin.

#### IV. DISCUSSION OF RESULTS

The experiments performed with the tissues of the frog, snake, rabbit, and rat, all agree in showing that no tissue has any power to absorb strophanthin, with the single exception of the frog's red blood corpuscles, and these can only take up very minute quantities of the drug; moreover no tissue has the power of

destroying strophanthin, except the snake's heart, which may possibly destroy a small proportion of any strophanthin that has been in contact with it for several hours. These results agree with those of Hatcher (14), but offer no support to the conclusion of Cloetta and Fischer (4) that the rat's heart can specifically absorb large quantities of cardiac glucosides.

The results obtained with the snake's heart may be compared with the experiments of Straub (23) with the heart of *Aplysia*: this organ is very insensitive to the action of most drugs, and Straub determined the relative distribution in the heart, and in the surrounding fluid, of veratrine, strychnine, curare, and atropine; he showed that veratrine in particular was stored in high concentrations by the heart muscle, and from these and other experiments he concluded that a drug, in order to act upon a tissue, must be stored by the tissue in a high concentration and must not be destroyed therein; from later work (24) upon the frog's heart, Straub concluded that strophanthin was an exception to this rule and that it produced its action without entering the heart muscle. The writer's experiments with strophanthin upon the frog's heart fully confirm Straub's last conclusion, and so also do the experiments upon the insensitive heart of the snake, in which the distribution of the drug could very readily be studied; even when the heart was killed with strophanthin, the concentration of the drug in the muscle was much lower than that in the perfusion fluid; there can therefore be no question of specific absorption of the drug by the tissue. The experiments upon mammalian hearts also showed that at most only traces of strophanthin were absorbed from the perfusion fluid.

It is possible that strophanthin produces its action by very small quantities entering into chemical combination with the muscle, in a form that cannot be recovered by extraction, but there is no evidence in favour of this view and it is much more probable that, as Straub (24) suggested, strophanthin acts by altering the physical condition of the surface membrane of the cells, without entering into chemical combination with any of the cell constituents.

Tolerance of strophanthin is due apparently in all cases to the

insusceptibility of those tissues upon which the drug usually exerts its action; this variety of tolerance offers an interesting contrast to the tolerance to atropine observed in the frog, and the rabbit, for the writer (3), and others, have shown that in this case tolerance is due to absorption and destruction of the drug by the liver, and other indifferent tissues. No tissue immunity exists, and any atropine which reaches the nerve endings affects them equally in the tolerant and in the susceptible animals. Therefore the method by which tolerance is established is totally different in the two drugs. The facts described above render difficult the explanation of the cumulative action of the cardiac glucosides. The observations of Hatcher and Bailey (15) upon the absorption of ouabain from the alimentary canal suggest that, in the therapeutic application of digitalis by the mouth, improvement in the circulation may increase the rate of absorption, and thus produce apparent cumulative effects; but this will not explain the cumulative action of subcutaneous doses of the cardiac glucosides, nor can this be explained by the slow absorption of these drugs from subcutaneous tissues (Fraenkel (9), von Lhota (19)): cumulative action therefore must be due, either to combination of strophanthin with heart muscle in quantities too small to be demonstrated, or to the drug "sensitising" the heart muscle in some way without forming any chemical combination, or else to small quantities of the drug remaining in the blood stream for prolonged periods after the greater part has been excreted; this last hypothesis is shown to be possible by experiment 14, when strophanthin was found in the blood of the snake sixteen hours after subcutaneous injection. Lhotak von Lhota (20) states that in addition to cumulative effects, a certain degree of tolerance can be produced in rabbits by administering cardiac glucosides for prolonged periods, he does not suggest in what manner this tolerance is established.

#### V. CONCLUSIONS

1. The common grass snake (*Tropidonotus natrix*) is very tolerant of strophanthin, this tolerance is due to tissue immunity, strophanthin having little effect upon the isolated heart; a similar



immunity, due to the same cause has been shown by other writers to exist in the toad and the rat.

2. There is no evidence that any tissue of the animals tested, (frog, snake, rabbit and rat) has any power to destroy strophanthin.

3. There is no evidence that the heart muscle of any of the above animals can specifically absorb strophanthin.

4. In the frog, rat and rabbit the liver has no power to absorb strophanthin.

5. The red blood corpuscles of the frog in the presence of plasma possess the power of absorbing small quantities of strophanthin, but the mammalian blood has no such power.

These experiments were performed in the Pharmacological Laboratories of Cambridge and University College, London, and the writer desires to express his thanks to Dr. Dixon and Professor Cushny for their kind advice and assistance. The expenses of this research were defrayed by a grant from the Royal Society.

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# THE PHARMACOLOGY OF THE SNAKE'S HEART

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The heart of the common grass snake (*tropidonotus natrix*), when isolated and perfused with frog's Ringer, will beat regularly and vigorously for as long as twenty-four hours, and, since it reacts to certain drugs differently from the frog's heart, it forms a preparation of considerable pharmacological interest. In the experiments described below isolated hearts were used, and were perfused by means of an apparatus described in a previous paper (1); a venous cannula was fixed in the inferior vena cava, and an arterial cannula in the left aorta, and all other vessels were ligatured. In all experiments the heart was perfused with Ringer supplied from a Marriotte's bottle. Records of the movements of the auricle and ventricle were taken by means of light straw levers writing on smoked paper, and the movements were magnified ten times. The Ringer used was of the following composition: NaCl 0.65 per cent; KCl 0.014 per cent; CaCl 0.012 per cent; NaHCO 0.02 per cent; NaHPO 0.001 per cent; Glucose 0.2 per cent. The heart, when first isolated and perfused with this mixture, often beat feebly but a strong regular beat always developed after half an hour.

## ACTION OF KATIONS UPON SNAKE'S HEART

Omission of calcium from the Ringer produced the same effects as in the frog's heart, namely a rapid diminution, in the strength of the beat, and of the rate of conduction in the heart, this last being measured by the length of the a-v interval. Lack of potassium produced prolongation of systole and incomplete dia-

stolic relaxation, but actual systolic arrest was not produced, even after prolonged perfusion.

#### ACIDS AND ALKALIES

The direct measurement of the acidity or alkalinity of fluids was found to be necessary in order to obtain values of even approximate accuracy. These measurements were made by means of Sørensen's indicator method (3), the standard solutions used were the phosphate and borate mixtures, and the following indicators were employed; p-nitrophenol, when the concentration of the hydrogen ion ( $C_{H^+}$ ) lay between  $10^{-5}$  and  $10^{-7}$ ; neutral red ( $C_{H^+}$   $10^{-7}$  to  $10^{-8.3}$ ); phenolphthalein, ( $C_{H^+}$   $10^{-8}$  to  $10^{-10}$ ); and tropäolin 0 ( $C_{H^+}$   $10^{-10}$  to  $10^{-12}$ ). Since  $C_{H^+} \times C_{OH^-}$  is a constant value, therefore the degree both of acidity and of alkalinity of fluids can be expressed by the molecular concentration of the hydrogen ion.

The Ringer used by the writer was found to have a  $C_{H^+}$  of  $10^{-8.3}$  and this appeared to be the optimum both for the snake's and the frog's heart. When acid fluids were perfused the snake's heart but little affected if the  $C_{H^+}$  was over  $10^{-6.0}$ , and a fluid with a  $C_{H^+}$  as high as  $10^{-5.8}$  did not produce diastolic arrest until after two hours. The frog's heart however was fairly rapidly arrested by fluids with a  $C_{H^+}$  below  $10^{-6.5}$ . The snake's heart moreover was distinctly less sensitive to alkalies than the frog's heart, in both hearts distinct systolic effects were produced by fluids with a  $C_{H^+}$  below  $10^{-9.5}$ .

The  $C_{H^+}$  of the serum of the snake and the frog were compared by means of the indicator method described by Walpole (5), the indicator used was neutral red, and in the snake's serum the  $C_{H^+}$  was found to lie between  $10^{-7.5}$  and  $10^{-7.8}$ , and in the frog's serum between  $10^{-7.2}$  and  $10^{-7.5}$ , the snake's serum being distinctly more alkaline. Mines (2) using the isolated hearts of the raia and scyllium found a similar difference in their susceptibility to alterations in the  $C_{H^+}$  of the perfusion fluid; the heart of the raia was as sensitive as that of the frog to increases in the  $C_{H^+}$ , but the heart of scyllium was much less sensitive; in this case also the serum of the less sensitive animal was the more alkaline.



FIG. 1. EFFECT OF ACID UPON ISOLATED SNAKE'S HEART<sup>1</sup>

*A*, after perfusion for one hour with normal Ringer ( $\text{CH}^+ = 10^{-7.7}$ ); *B* and *B'*, after perfusion twenty minutes with faintly acid Ringer ( $\text{CH}^+ = 10^{-6.9}$ ); *C*, after perfusion forty minutes with acid Ringer ( $\text{CH}^+ = 10^{-5.8}$ ); *D*, after perfusion two hours with acid Ringer ( $\text{CH}^+ = 10^{-5.3}$ ).

<sup>1</sup> In all figures the upper tracing shows auricular movements and the lower tracing ventricular movements, the movements are magnified ten times by the levers used. Read from left to right. Time in seconds.

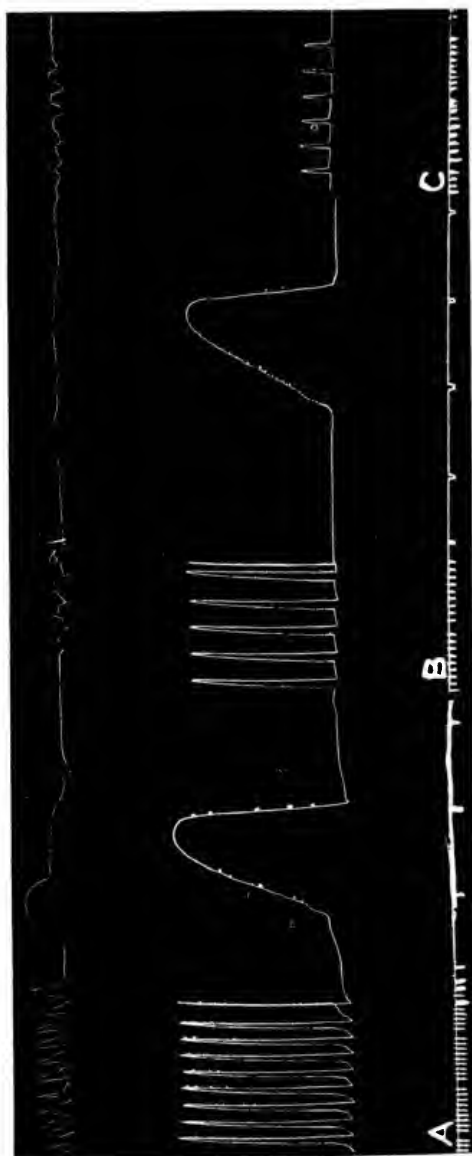


FIG. 2. EFFECT OF ACID UPON ISOLATED FROG'S HEART

A, perfused with normal Ringer ( $\text{CH}^+ = 10^{-7.1}$ ); B, after perfusion twenty minutes with faintly acid Ringer ( $\text{CH}^+ = 10^{-6.5}$ ); C, after perfusion eighty minutes with faintly acid Ringer ( $\text{CH}^+ = 10^{-6.5}$ ).

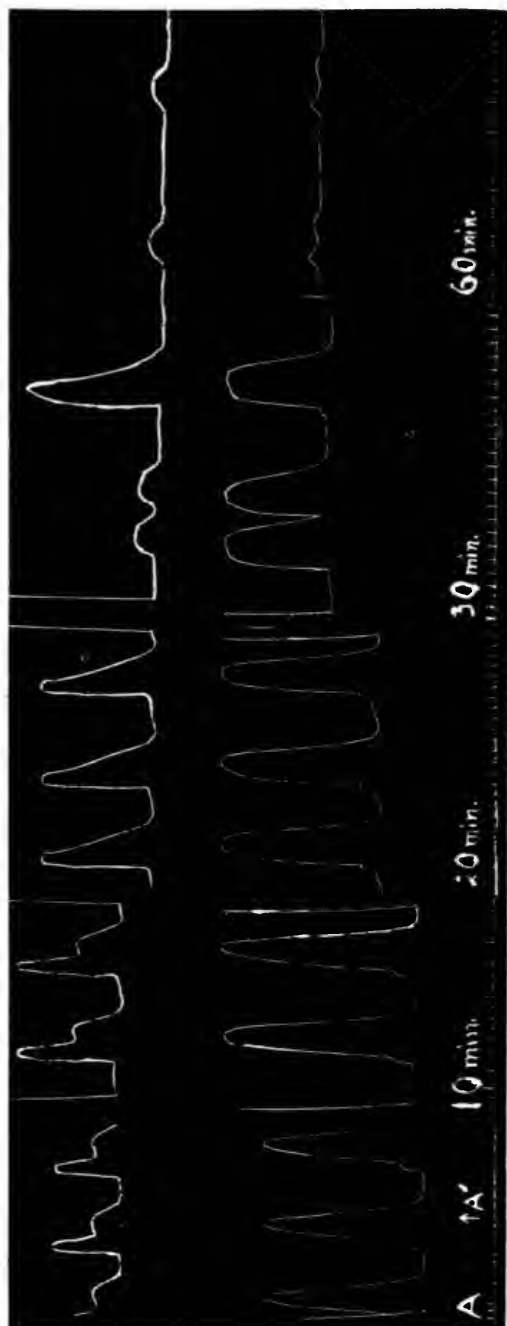


FIG. 3. ACTION OF STROPHANTHIN UPON ISOLATED SNAKE'S HEART

A, perfused normal Ringer one hour; A', strophanthin 0.2 per cent introduced

## STROPHANTHIN

The snake's heart was found to be extraordinarily insensitive to strophanthin, for, with the strophanthin used (Wellcome brand

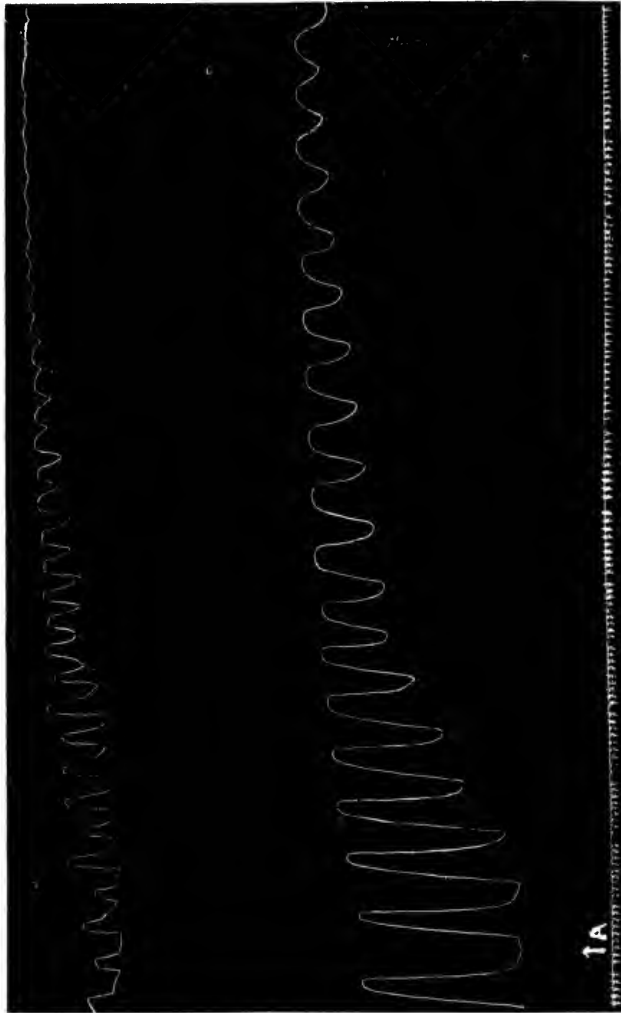


FIG. 4. ACTION OF BARIUM UPON ISOLATED SNAKE'S HEART

A, barium chloride 0.1 per cent introduced

B. & W.), a concentration of 0.0005 mg. per. cubic centimeter killed a frog's heart within two hours, but a concentration of



2.0 mg. per cubic centimeter was required to produce this effect in a snake's heart, and the heart was apparently unaffected by concentrations below 0.5 mg. per cubic centimeter.

#### DISCUSSION

The writer has shown (1) that this tolerance of the snake's heart to strophanthin is not due to destruction of the drug by the heart, and also it is not associated with the presence in the



Fig. 5. ACTION OF SAPOTOXIN UPON ISOLATED SNAKE'S HEART

A, sapotoxin 0.5 per cent introduced

snake's blood of any body with an action resembling that of the cardiac glucosides. The tolerance to strophanthin is moreover not associated with any general tolerance to drugs that produce systolic arrest of the heart. Barium chloride 0.1 per cent produced rapid systolic arrest of the snake's heart, and a similar concentration was required to produce the same effect in a frog's heart: sapotoxin 0.02 per cent also produced rapid systolic arrest of the snake's heart, and about the same concentration was required to produce arrest of the frog's heart. Since the snake's heart is more than 1000 times as tolerant of strophanthin as the frog's heart it is clear that both barium and saponin must act upon the heart in an entirely different manner to strophanthin.

TABLE 1.

*The molecular concentrations of certain drugs that will just kill within an hour the isolated hearts of the snake and the frog*

DRUG	SNAKE'S HEART	FROG'S HEART
i. Alkali (concentration of OH <sup>-</sup> ).....	0.0005	0.0002
ii. Acid (concentration of H <sup>+</sup> ).....	0.000003	0.0000006
iii. Barium chloride.....	0.005 to 0.0025	0.005 to 0.0025
iv. Sapotoxin (mol. wt. 408).....	Below 0.0005	Above 0.0002
v. Strophanthin (mol. wt. 922).....	0.0022	0.000001

As shown in the table the tolerance to strophanthin is associated with a well marked tolerance of alterations in the concentration of the hydrogen ion in the perfusion fluid. Mines (2) found that, in the heart of scyllium, a tolerance to increase in the  $C_{H^+}$  was associated with tolerance of the tri-valent kations (lanthanum etc.); he suggested that the activity of the heart is dependent upon the maintenance of a certain surface potential upon those surfaces within the heart that are semi-permeable to ions; any such potential existing on the surface of an animal membrane in contact with weak alkali is increased when the  $C_{H^+}$  is decreased, and is diminished when the  $C_{H^+}$  is increased, but the rate and extent of this diminution also depends upon the nature of the membrane. Mines shows that the tri-valent kations

probably act by altering the nature of the heart membranes in such a way that the surface potential is diminished although the  $C_{H^+}$  of the surrounding fluid remains constant. The membranes in the heart of scyllium do not readily lose their surface potential and therefore the heart is tolerant of acids (increase of  $C_{H^+}$ ), and still more tolerant of tri-valent kations.

I have shown in a previous paper that, as suggested by Straub (4), the action of strophanthin is probably of a physical nature, and that the drug acts upon the surface of the heart without entering the cells, and since, in the snake's heart, tolerance to strophanthin is associated with a distinct tolerance to alterations in the  $C_{H^+}$  of the perfusion fluid, it appears probable that the action of strophanthin is due to it modifying the surface potential existing upon the membranes of the heart.

#### CONCLUSIONS

1. The isolated snake's heart reacts to barium and saponin in the same manner as the frog's heart.

2. The isolated snake's heart is less readily affected than the frog's heart by alterations in the concentration of the hydrogen ion in the perfusion fluid.

3. The isolated snake's heart shows a very marked tolerance to strophanthin.

4. These facts show that there is no close relation between the action of strophanthin and that of certain other drugs that produce systole (e.g., barium and saponin).

5. It appears probable that the action of strophanthin is connected with the power that a heart possesses to resist changes in the concentration of the hydrogen ion, and that the action is of a physical nature.

The expenses of this research were defrayed by a grant from the Royal Society.

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## THE RELATION OF VASCULAR CONDITIONS TO PITUITRIN DIURESIS

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Despite considerable investigation of the matter, opinion is still divided as to whether the diuretic effect of pituitary extract is due to circulatory changes or to an influence exerted upon the renal cells directly. Schaefer and Herring (1) who have made probably the most extensive study of the matter were able to find no constant relation between diuresis and changes in either general blood pressure or kidney volume. Although the injection of pituitary extract typically results in higher blood pressure, kidney dilation and diuresis, instances were observed in which a "repeat dose" produced greater urine flow without change of blood pressure. Likewise in certain cases marked diuresis was seen when there was no increase in kidney volume. They concluded, therefore, that pituitary diuresis is due primarily to direct stimulation of the renal cells. Houghton and Merrill (2) on the other hand, after a series of perfusion experiments on the isolated kidney, reached the opposite conclusion that the diuresis is due primarily to vascular changes. Results obtained in an isolated organ, however, are less conclusive as regards normal functioning than those obtained in the animal directly.

So far as increased blood pressure is concerned this is a much less important factor in diuresis than was long supposed. On *a priori* grounds one would suppose that a rise in arterial tension would increase the blood flow through the kidney and thus promote diuresis. Actual experimentation, however, does not bear out the supposition. Burton-Opitz and Lucas (3) have recently made a careful study of this point. After finding the

rate of blood flow through the kidneys in an animal, systemic pressure was experimentally increased and the rate of flow again determined. In the greater number of their experiments there was no increase even after a high arterial tension had been secured. Indeed in some cases they noted a decrease in flow.

A number of investigators have made more direct studies of the relation of changes of blood pressure to kidney secretion itself. Erlanger and Hooker (4) using Erlanger's sphygmomanometer made a series of careful observations upon two men—one perfectly healthy and the other having albuminuria. They found that changes in general blood pressure, either systolic or diastolic had in themselves little or no effect upon urine secretion. Increased pulse pressure on the other hand, did materially promote diuresis. Hooker (5) later carried out a set of similar experiments upon isolated kidneys directly and was led to the same conclusion. Lawrence (6) has recently made an extensive clinical study of the relation between vascular hypertension and urinary secretion. He reached the same conclusion as did Erlanger and Hooker that there is no relation between systolic or diastolic pressures and diuresis. He found further, however, that whenever a falling systolic pressure and rising pulse pressure occurred together, an increased urine flow resulted. He reached the general conclusion that whenever, after being disturbed, the pulse and systolic pressures are approaching the normal ratio 1 to 3, an augmented renal secretion occurs.

In the light of the foregoing observations it seemed desirable to investigate further the simultaneous effects of pituitrin upon urinary secretion and vascular conditions with particular attention to pulse pressures.

In all our experiments dogs were employed. For pituitary material the commercial "Pituitrin" of Parke Davis and Company was utilized. Ether-urethane anesthesia was used. The animal were first etherized and fastened in the holders then urethane, about 1 gram per kilo, was given by stomach; as the urethane became effective the ether was gradually discontinued. Carotid blood pressure was recorded by means of an ordinary mercury manometer and float. Urine output was recorded by

drops as it flowed from a uretral cannula. Pulse pressure was recorded from the other carotid artery by means of a plethysmograf and tambour. The artery was dissected free throughout the length of the neck. Then it was cut between two ligatures as far cephalad as possible. After retraction there remained exposed a segment of artery 4 or 5 cm. long which changed volume with every variation of internal pressure. The plethysmograf was made by fastening a delicate rubber diaphragm over one end of a segment of glass tube which fitted loosely over the artery segment. A perforation slightly smaller than the cross-section of the artery was made in the diaphragm. By means of the ligature at the distal end the cut artery was drawn through the diaphragm and secured in the plethysmograf by the same ligature which was bound to the outside by slipping on a rubber tube. The other end of the tube connected with a delicate tambour. The arrangement afforded a satisfactory tracing of pulsations in the artery and a delicate record of variations in pulse pressure. A slight leak in the system prevented changes of abscissa level with variations of general blood pressure. No attempt was made to calibrate the apparatus since only comparative observations were desired.

Pituitrin injections were made under various conditions of blood pressure and in varying dosage from 0.3 cc. to 1.3 cc. Usually the drug was introduced into a femoral vein but in a few instances subcutaneous injections were made use of.

Contrary to our expectations there was found absolutely no demonstrable relation between pulse pressure and urine flow. The most common condition for diuresis was an augmented systolic together with a decreased pulse pressure. In such cases the ratio of pulse pressure to systolic pressure was notably smaller than normal but instances were observed in which increasing flow occurred with increasing as well as with diminishing pulse pressures. So far as general blood pressure is concerned Schaefer and Herring's results were confirmed. "Repeat doses" sometimes produced diuresis without increased blood pressure, occasionally increased pressure occurred without diuresis and often diuresis increased as general pressure fell.

In figures 1, 2 and 3 are reproduced some of the significant records obtained. In figure 1 a "repeat dose" of pituitrin (0.3 cc. intravenously) caused a diuresis accompanied by a rise of blood pressure and a marked decrease of pulse pressure. Figure 2 (*a*) shows a case in which the pituitrin (0.7 cc. intravenously) caused first a sharp rise of general blood pressure and fall of pulse pressure with suppression of urine for three minutes. Figure 2 (*b*) shows conditions twelve minutes after the injection. The diuresis was at its height after systolic pressure had returned

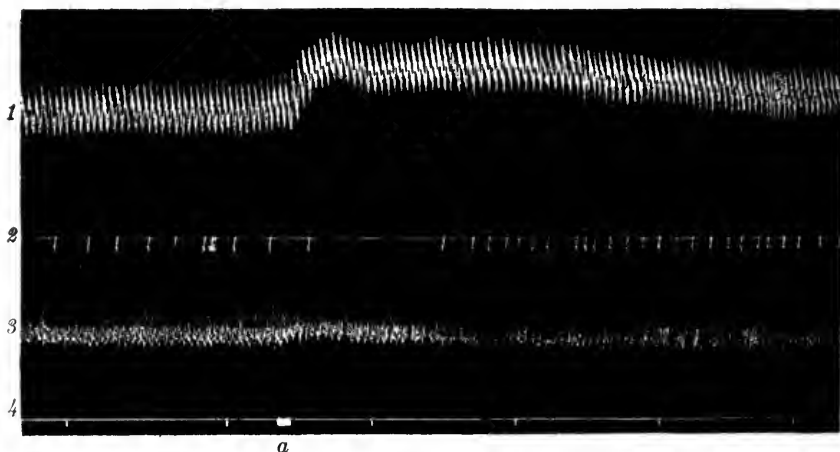


FIG. 1. At *a* 0.3 cc. pituitrin injected intravenously ("repeat dose"). 1, carotid blood pressure (130 mm.); 2, urine registered by drops; 3, carotid pulse; 4, time, one minute.

to normal but while pulse pressure was still materially depressed. Figure 3 shows an instance in which the drug (1.0 cc. subcutaneously) caused a sharp increase of pulse pressure but left general blood pressure and urine flow unaffected. In this instance the pulse pressure reached such amplitude that the stroke was not all recorded. In the instances figured, then, there occurred (1) diuresis with systolic pressure raised and pulse pressure decreased; (2) diuresis with systolic pressure normal and pulse pressure decreased; (3) urinary suppression with systolic pressure increased and pulse pressure decreased and (4) systolic pressure normal



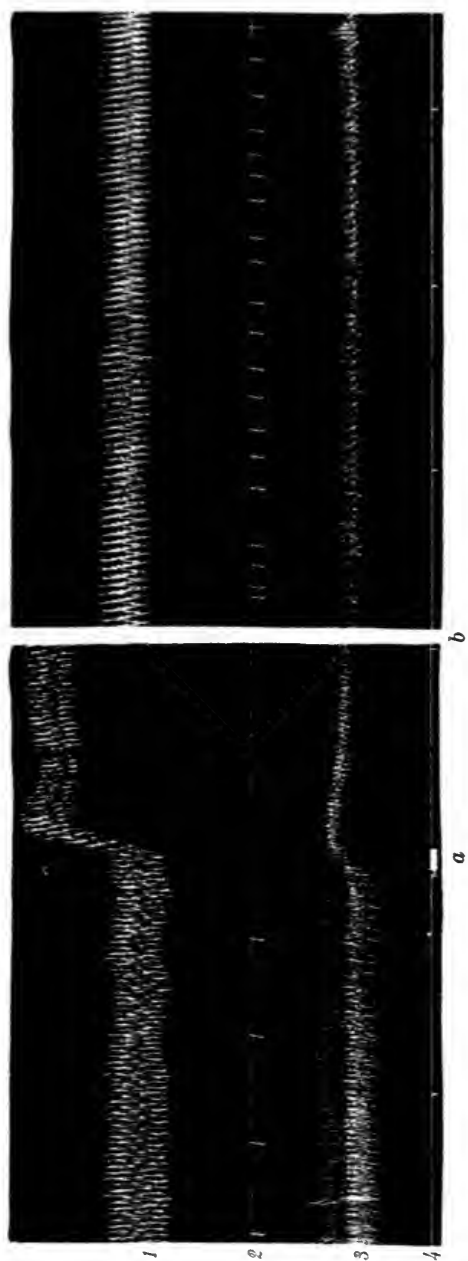


FIG. 2. At *a* 0.7 cc. pituitrin injected intravenously. 1, carotid blood pressure (120 mm.); 2, urine registered by drops; 3, carotid pulse; 4, time, one minute. At *b* record for eleven minutes omitted.

and pulse pressure increased without diuresis. Various other combinations might have been shown.

The foregoing observations as a whole indicate that, as Schaefer and Herring maintained, pituitrin exerts its diuretic effect not primarily by altering vascular conditions but by stimulating the renal cells directly. So far as blood flow through the kidney is

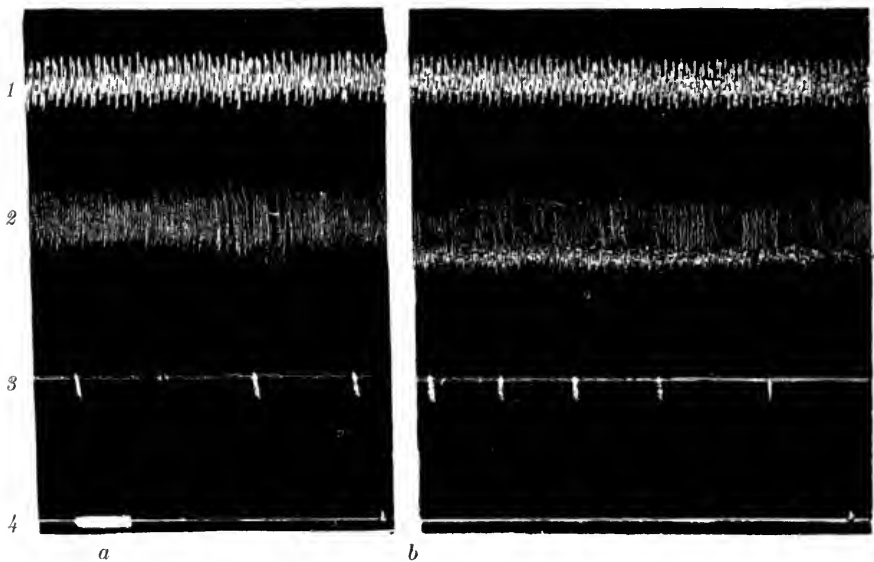


FIG. 3. At *a*, 1 cc. pituitrin injected subcutaneously; at *b*, two minutes record omitted. 1, carotid blood pressure; 2, carotid pulse; 3, urine registered by drops; 4, time, signal and 0 pressure line. (In *b*, 2, full amplitude not registered.)

concerned the case seems to be analagous with that of the salivary glands in which increased blood flow usually accompanies and favors increased activity but does not cause it. On the other hand when any tendency to secrete is interfered with by abnormal vascular conditions a return toward normal will permit the expression of this tendency and may seem, therefore, actually to cause secretion.

## SUMMARY

1. Pituitrin in suitable dosage causes diuresis in the anesthetized dog.

2. There is no constant relationship between pituitrin diuresis and either systolic pressure or pulse pressure or the ratio between them, although

3. Pituitrin diuresis usually is accompanied by decreased pulse pressure.

Previous researches (Schaefer and Herring) have shown that there is no constant relationship between changes of kidney volume and pituitary diuresis.

4. Pituitrin diuresis, therefore, is due primarily to direct stimulation of the renal cells—usually aided, probably, by a concomitant vasodilation in the kidneys.

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(2) Houghton and Merrill: *Jour. Am. Med. Assoc.*, 1908, li, p. 1849.

(3) Burton-Opitz and Lucas: *Jour. Expt. Med.*, 1911, xiii, p. 308.

(4) Erlanger and Hooker: *Johns Hopkins Hosp. Rep.*, 1904, xii, p. 145.

(5) Hooker: *Am. Jour. Physiol.*, 1910, xxvii, p. 24.

(6) Lawrence: *Am. Jour. Med. Sc.*, 1912, cxiv, p. 330.



# SALINE PERFUSION OF THE RESPIRATORY CENTER IN FROGS: THE EFFECT OF CALCIUM CHLORIDE AND POTASSIUM CHLORIDE

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## INTRODUCTION

So far as I have been able to find no one has attempted to study by direct perfusion the effect of the inorganic salts of the blood upon the rhythmic discharge of the respiratory center. The fact that these salts exert a striking influence over other rhythmic tissues, i.e., the heart, the smooth muscle from the pharynx of frogs<sup>1</sup> and the cilia of marine animals<sup>2</sup> has been abundantly demonstrated, and Carlson<sup>3</sup> has investigated their effect upon the rhythmic heart ganglia of limulus. The last mentioned work bears more directly upon the present experiments because it deals with a rhythmic nervous mechanism. Carlson finds that potassium exerts a primary stimulating effect which is followed by depression, while calcium causes depression without primary stimulation. My results agree with these findings so far as potassium is concerned when this salt is increased in amount (transitory excitation followed by depression), but in the case of calcium, I have noted a uniform excitation when the salt is increased in amount.

## METHODS

The experiments were performed upon frogs. The brain was transected anterior to the optic lobes and the spinal cord crushed in the brachial region. The abdominal wall was then freely

<sup>1</sup> Stiles: American Journal of Physiology, 1901, v, 338.

<sup>2</sup> Mayer: Carnegie Institution of Washington, Publication No. 132, p. 3, 1911.

<sup>3</sup> Carlson: American Journal of Physiology, 1906, xvi, 386.

opened, the venous base of the heart cut away and a canula tied into the bulbus arteriosus. Through this canula the solutions to be investigated were perfused at a pressure of approximately 30 cm. of water. The solutions were in all cases saturated with oxygen and their perfusion pressures so adjusted that their rate of flow was uniform. This precaution was called for because otherwise the variation in oxygen supplied to the respiratory center incidental to the vascular tone effect of calcium and potassium<sup>4</sup> disturbed the results. As soon as the artificial circulation was established, the respiratory muscles of the throat were connected with a recording lever.

In the great majority of cases, the interruption of the circulation by the operation had no bad effect upon the center so that the record could be begun almost at once. In others the respiratory paralysis due to anaemia did not pass off for several minutes. The record of movements thus obtained was produced by the rhythmic discharge of the respiratory center because (1) destruction of the medulla caused them to cease, (2) substitution of carbon dioxide for oxygen in the perfusion fluid brought about a few violent respiratory movements followed by inspiratory spasm which in turn gave place to relaxation and complete cessation of movement, and (3) stoppage of perfusion produced dyspnoeic movements.

Herlitzka<sup>5</sup> found that an oxygenated solution made up of NaCl 0.6, CaCl<sub>2</sub> 0.01, KCl 0.0075, NaHCO<sub>3</sub> 0.01, dextrose 0.1 and urea 0.2 per cent when perfused in frogs would maintain the reflex irritability of nerve centers for eight hours. In the present experiments I have used a solution of NaCl 0.7, CaCl<sub>2</sub> 0.03, KCl 0.03, dextrose 0.1 and urea 0.2 per cent. With such a solution the respiratory center will continue to discharge rhythmically for four hours or more. No effort was made to obtain an optimum mixture first because of the problem as defined and second because the mixture hit upon was in use before reference was made to Herlitzka's work. The fact observed that the center continues active for a shorter time when it is exposed to experi-

<sup>4</sup> Hooker: *American Journal of Physiology*, 1911, vi, 361.

<sup>5</sup> Herlitzka: *Archivio di Fisiologia*, 1909, vi, 369.

mental mixtures than when fed continuously with the normal or control mixture further lessens the particular need of finding the most perfect solution. In experimental practice the records were never continued for more than two hours, and in most cases for less than one hour.

Since the center responded most normally when perfused with a solution containing both calcium and potassium, the first experiments were directed to a study of increasing or decreasing the amount of one of these salts in the perfusion fluid and comparing the results obtained with the normal record from the same frog. The solutions used were made up in parts of one hundred. Although, under these conditions, there is obviously some variation in the molecular concentration of the fluid it seems unlikely that such variation is sufficient to be a factor in determining the results.

It will be noted that the experimental procedure resulted in the perfusion of the muscles from which the record was obtained. In consequence of this, no conclusions can be drawn from the amplitude of the respiratory movements as to the force of discharge by the respiratory center because Mines<sup>6</sup> has shown that the height of contraction of frogs' sartorius muscle under rhythmic stimulation is markedly influenced by the composition of the fluid bathing the muscle under observation. He found that the addition of a small amount of KCl (0.05 per cent) to normal saline increased the response while a large amount of KCl (0.1 per cent) inhibited the response completely. On the other hand the addition of  $\text{CaCl}_2$  0.05 per cent decreased the response. The effect of the latter salt in greater concentration was not discussed by him. These facts, do not, however, bear upon the rate of discharge of the respiratory center as reported in this paper, nor do they accord entirely with the records here produced so far as amplitude is concerned. In order to remove the possible objection that the salts, by direct action upon the muscles might account for the records obtained and so leave their action upon the respiratory center in doubt, two experiments were performed in which the recording muscles were excluded from the perfusion. This

<sup>6</sup> Mines: *Journal of Physiology*, 1908, xxxvii, 408.

was accomplished by ligation of the external carotid close to the carotid gland, the laryngeal and the pulmono-cutaneous arteries. Subsequent injection of india ink demonstrated the efficacy of the ligatures. To maintain the vertebral supply to the medulla (apparently essential in the frog) the aortic arches were not ligated thus leaving the superficial muscles of the throat to be perfused by way of the occipital branches. These muscles were consequently cut away and excluded from the record. The results in these two experiments agreed entirely with the results from the other experiments.

#### EXPERIMENTAL

*The influence of varying amounts of calcium.* In each case comparison was made with the normal solution in which 0.03 per cent of calcium chloride was present. The amounts investigated were 0.015, 0.045, 0.06 and 0.12 per cent. The results of this series indicate that calcium, in the presence of potassium, is a stimulant. For when the amount of calcium was reduced the center was depressed and when the amount was increased the center was stimulated. This effect is demonstrated in figure 1 which exhibits the effect of increasing the calcium chloride to 0.06 per cent. Fourteen observations were devoted to this point in all of them the results were consistent. In addition two experiments were directed to a study of the behavior of the center when the amounts of calcium and potassium chloride in the control solution were doubled (i.e. 0.06 per cent). This solution was then compared with one in which the calcium chloride was 0.03 per cent. The effect of the latter solution was to depress the center.

*The influence of varying amounts of potassium.* The results here are the exact converse of those noted above for calcium. The experiments were paralleled throughout and in a number of cases the same frog was used in studying the influence of both of the salts. Figure 2 exhibits the depressant action of the solution in which potassium chloride has been increased to 0.06 per cent.



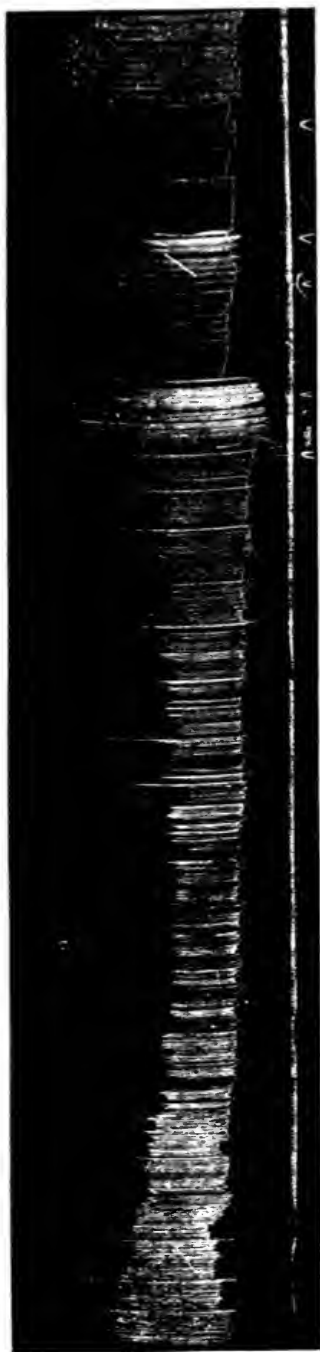


FIG. 1. THE EXCITANT EFFECT OF AN INCREASE OF  $\text{CaCl}_2$

Between the pairs of arrows and after the last arrow the control solution (containing  $\text{CaCl}_2$  0.03 per cent) was replaced by a similar solution containing  $\text{CaCl}_2$  0.06 per cent. During the long preliminary period the center was recovering from a prolonged perfusion with the modified solution.



FIG. 2. THE DEPRESSANT EFFECT OF AN INCREASE OF  $\text{KCl}$

Between the pairs of arrows the control solution (containing  $\text{KCl}$  0.03 per cent) was replaced by a similar solution containing  $\text{KCl}$  0.06 per cent.

*The influence of omitting calcium.* In this series the normal solution was compared with one from which the calcium chloride was omitted. Under such conditions the effect observed might be regarded as due either to the absence of calcium or to the preponderating influence of potassium. That the latter is presumably the factor involved will appear from the later experiments. The removal of the calcium chloride from the perfusion fluid uniformly results in increased activity of the center. This is shown in figure 3 and was demonstrated on ten different preparations.

*The influence of omitting potassium.* As in those experiments directed to study the influence of varying the amount of one or the other salt, so here it is found that potassium produces an effect the exact converse of that of calcium. The removal of the potassium results in a decided depression of the respiratory center. This is shown in figure 4 and was also demonstrated on numerous other preparations.

*The influence of increasing the amount of one salt in the absence of the other.* If the potassium be omitted and at the same time the amount of calcium be increased (i.e., 0.06 per cent) then instead of depression as above noted excitation of the center is produced. And conversely omission of calcium and increase of potassium (i.e., 0.06 per cent) results a depression of center.

The experiments thus far presented may be tabulated with respect to the normal or control solutions as follows:

<i>Stimulation</i>	<i>Depression</i>
Increase of calcium,	Increase of potassium,
Decrease of potassium,	Decrease of calcium,
Omission of calcium,	Omission of potassium,
Increase of calcium in absence of potassium.	Increase of potassium in absence of calcium.

These results point to the fact that calcium in small amount depresses and in large amount stimulates while with potassium small amounts stimulates and large amounts depresses. It appeared desirable, however, to attempt to analyse the effects of calcium and potassium each independently of the other. Accordingly the control solution was made to contain dextrose 0.1, urea 0.2,



FIG. 3. THE EXCITANT EFFECT OF REMOVAL OF  $\text{CaCl}_2$

Between the first two arrows and after the last arrow the control solution (containing  $\text{CaCl}_2$  0.03 per cent) was replaced by a similar solution from which  $\text{CaCl}_2$  was omitted.



FIG. 4. THE DEPRESSANT EFFECT OF REMOVAL OF  $\text{KCl}$

Between the pairs of arrows the control solution (containing  $\text{KCl}$  0.03 per cent) was replaced by a similar solution from which  $\text{KCl}$  was omitted.

and NaCl 0.7 per cent. That such a perfusion fluid is unsatisfactory is evident in figure 5 in which it is compared with a solution containing both calcium and potassium. Under the influence of such a solution the center discharges irregularly and its vitality is very materially lessened. It is open to question, therefore, whether the results of this series are of definite value. They appear to substantiate the view that small amounts of potassium (i.e., 0.03 per cent) and large amounts of calcium (i.e., 0.06 to 0.12 per cent) are stimulant in their effect. But so far as the depressant

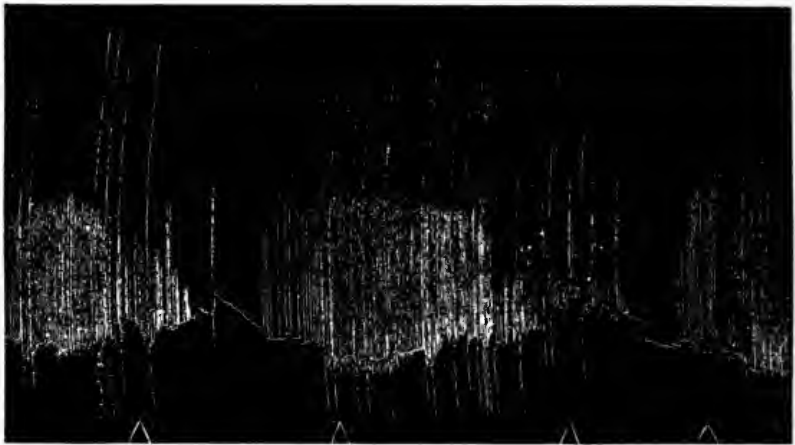


FIG. 5. THE DEPRESSION OF RESPIRATORY RHYTHM IN THE ABSENCE OF  $\text{CaCl}_2$  AND KCl

Between the pairs of arrows the control solution, containing  $\text{CaCl}_2$  0.03 per cent and KCl 0.03 per cent, was replaced by a similar solution from which these salts were omitted.

action of these salts is concerned they are of decidedly uncertain value. And even in the case of their stimulating action these results alone would not be regarded as convincing.

#### CONCLUSIONS

The respiratory center in the frog may be perfused with a suitable salt solution and will continue to discharge for several hours.

The preparation has been used to study the influence of calcium and potassium with the following results:

1. Using a salt solution, balanced with respect to calcium and potassium, removal of potassium, causes depression, and removal of calcium causes excitation of the center.

2. Using such a balance solution an increase of potassium causes depression, and a decrease causes excitation. On the other hand an increase of calcium causes excitation and a decrease causes depression.

3. Calcium and potassium are essential to the normal functioning of the preparation. In their absence the discharge of the center is irregular and its viability much reduced.

4. The addition to a calcium-potassium-free solution of a small amount of potassium (0.03 per cent) or a large amount of calcium (0.06 per cent) *may* excite the center. It could not be satisfactorily shown in this case that small amounts of calcium or large amounts of potassium have a depressant action.



## THE ABSORPTION AND EXCRETION OF AMMONIA BY THE LUNGS

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Thiry<sup>1</sup> was one of the first workers on this problem who used a method to exclude the mouth and upper respiratory passages. He inserted a tracheal cannula in rabbits and after removing the carbon dioxide by absorption in strong sodium hydrate passed the expired air through Nessler's solution to detect the ammonia. He found that traces of ammonia were excreted by the lungs. Lossen and Voit,<sup>2</sup> also Kühne and Strauch,<sup>3</sup> confirmed this result using rabbits and cats. Bachl<sup>4</sup> several years later repeated the work with extreme care but failed to detect ammonia in the expired air. He found sufficient ammonia in the reagents to account for the findings of the previous workers. He also quotes Voit as having confirmed his result. Still later Schiffer<sup>5</sup> also failed to find ammonia in the expired air of rabbits. Apparently then the excretion of ammonia by the lungs is either extremely minute in amount or absent entirely.

Previous to this time the older workers,<sup>6</sup> Marchand 1844, Thompson, Reuling, Guterbock, Richardson, Widerhold, Shottin, Gorup Besanz, Mettenheimer and others, all found ammonia in the respired air but they did not exclude the possibility of its coming from the mouth or upper respiratory tract. Reuling noted that the excretion of ammonia was greater in those with

<sup>1</sup> Thiry: *Zeitschrift für Rationelle Medizin*, 1863, xvii, p. 166.

<sup>2</sup> Lossen: *Zeitschrift für Biologie* 1865, i, p. 210.

<sup>3</sup> Kühne and Strauch, quoted by Bachl: *Centrallblatt für die medizinische Wissenschaft*, 1862, No. 37, s. 578.

<sup>4</sup> Bachl: *Zeitschrift für Biologie*, 1869, v, p. 61.

<sup>5</sup> Schiffer: *Berliner Klinische Wochenschrift*, 1872, No. 42, p. 508.

<sup>6</sup> References for these are given by Thiry and Lossen, loc. cit.

carious teeth. Guterbock found it much smaller in those with sound teeth. Regnault and Reiset<sup>7</sup> determined the amount and reported that about 10 mgm. were respired by rabbits and 15 by dogs in twenty-four hours. However they found about the same amount in the respired air of the same period. The traces, therefore, which were found, were within the limits of accuracy of any available method, and it remained questionable whether any ammonia was excreted directly through the lungs from the blood.

Pettenkofer and Voit<sup>8</sup> confirmed the work of Regnault and Reiset; the amount of ammonia they found was not large enough for quantitative determination and was too small to be of any importance in metabolic work. The question therefore arises: Are the lungs permeable to ammonia?

Spence<sup>9</sup> states that Blake injected ammonia hydrate into the veins of an animal and found ammonia excreted by the lungs. Boehm and Lange<sup>10</sup> were unable to find ammonia in the expired air of cats after the injection of ammonium salts. Knoll<sup>11</sup> found no symptoms to indicate the absorption of ammonia when vagotomized rabbits were allowed to breath strong ammonia through a tracheal cannula. Salaskin<sup>12</sup> could not detect ammonia in the exhaled air after an Eck fistula operation when the amount of ammonia in the blood is said to be increased. He also reports to have found 2.56 mgm. of ammonia in the saliva when only 1.12 mgm. were present in the blood. Henderson<sup>13</sup> questions the accuracy of this statement and we have been unable to corroborate it. Magnus<sup>14</sup> states that the lungs are impermeable to ammonia.

<sup>7</sup> Regnault and Reiset: *Annalen der Chemie und Pharmacie*, 1850, lxxiii, p. 92.

<sup>8</sup> Pettenkofer and Voit: *Annalen der Chemie und Pharmacie*, 1862, supplement volume ii, p. 59; also *ibid.*, p. 361.

<sup>9</sup> Spence: *Edinburgh Medical Journal*, 1866, xii, p. 44.

<sup>10</sup> Boehm and Lange: *Archiv. f. experimentelle Path. u. Pharma.*, 1874, ii, p. 364.

<sup>11</sup> Knoll: *Sitzungs-Bericht der Wiener Akademie*, 1873, No. 68, iii, p. 245.

<sup>12</sup> Salaskin: *Zeitschrift f. Physiologische Chemie*, 1898, xxv, p. 463.

<sup>13</sup> Henderson: *Journal of Pharmacology*, 1910, ii, p. 5.

<sup>14</sup> Magnus: *Archiv. f. Experimentelle Pathologie u. Pharmacologie*, 1902, Bd. xlviii, p. 100.



This view, though adopted by many textbooks,<sup>15</sup> is incorrect as shown below.

Amoss<sup>16</sup> has recently found ammonia in the expired air but believes that it comes from the decomposition of food particles about the teeth, a view in harmony with, and perhaps based upon, the work of Reuling, Guterbock, and the latter work of Lehmann and Jessen,<sup>17</sup> as well as that of Bergey;<sup>18</sup> all of whom found varying amounts of ammonia in the breath, and more in those with unsound teeth. Bergey also found ammonia in the breath of a man with a tracheal fistula. Formanek<sup>19</sup> also believes the ammonia in the expired air of sound or tracheotomized animals is a product of local decomposition and does not pass through the lungs.

The difference in the opinions quoted may be due to several causes. An animal unassisted can inspire against a comparatively small pressure, consequently to wash all the ammonia out of the inspired air is rather difficult, due mainly to the rapidity with which the air enters the lungs. For the same reason the air is expelled with such force and volume that it is difficult to remove all the carbon dioxide by absorption. If this be not removed and it passes through Nessler's solution it decomposes and renders the Nessler reagent incapable of demonstrating ammonia. It is quite probable that this has happened in the majority of the cases of failure to detect ammonia in the expired air. To remove all the ammonia from the inspired air I used compressed air and passed this through several bottles of sulphuric acid using Folin ammonia tubes to break it and allow absorption. It was then passed through a Nessler solution before it entered the lungs. The exhaled air was passed through several bottles of dilute acid (normal one-fifth) to absorb any ammonia that might be expired. This was boiled and neutralized with NaOH before Nesslerizing. In this way a long series of absorption tubes could be

<sup>15</sup> Cushny, Sollman, Lusk: Textbooks.

<sup>16</sup> Amoss: *Journal of Experimental Medicine*, 1913, xvii, p. 149.

<sup>17</sup> Lehmann and Jessen: *Archiv. fur Hygiene*, 1890, x, p. 367.

<sup>18</sup> Bergey: *Smithsonian Annual Report* 1895, p. 389.

<sup>19</sup> Formanek: *Archiv. f. Hygiene*, 1900, xxxviii, p. 1.

used and the compressed air made it possible for the animal to respire without hindrance. The use of strong sodium hydrate or barium hydrate was tried to absorb the exhaled carbon dioxide but I have more faith in the former method for the reasons given. The conclusion is that traces of ammonia are normally excreted by the lungs. This opinion is strengthened by the absolute certainty that ammonia is absorbed by the lungs as shown by the onset of spasms, also by the analysis of the blood before and after the inhalation of ammonia, and also by the excretion of ammonium salts into the lung when injected into the vein. There can therefore be no question that the lungs are permeable to ammonia. Why mere traces are excreted while large amounts may be absorbed undoubtedly lies in the chemistry of the lung tissue and also in the changes which ammonia readily undergoes in the blood.

Most volatile substances like the sulphides pass through the lungs with amazing rapidity. If a solution of sulphide be injected into the veins of an animal it can be detected easily in the breath in a very few seconds. Such rapidity of excretion and ease of detection does not occur in the case of ammonia. While ammonia passes through it takes a much longer time to detect it. In repeating Knoll's experiment, Magnus allowed a vagotomized rabbit to inhale ammonium hydrate, 70 to 1000, through a tracheal cannula for three minutes, and failed to notice any symptoms of absorption. This observation we have repeated and confirmed, but this does not mean that there was no absorption, but more probably that the absorption was slow or that the absorbed ammonia changed with such rapidity that symptoms did not develop during three minutes.

The following protocols show that ammonia is absorbed and excreted by the lungs:

*March 15, 1913.* To show the absorption of ammonia. Rabbit, 1500 grams.

9.30 Given two grams of urethane by mouth.

10.30 Etherized, both vagi cut and a tracheal cannula inserted.

10.55 Commenced to breathe through ammonium hydrate 70 : 1000.

- 11.00 Respiration distinctly stimulated. Much mucus and a trace of blood in the cannula.
- 11.05 Respiration labored.
- 11.06 28 per cent ammonia substituted.
- 11.10 Distinct ammonium tetanus. All muscles involved.
- 11.12 Animal dies from paralysis of the respiration.

*March 28, 1913.* To show the absorption of ammonia by the lungs. A dog 10 kilos weight was given a large dose of morphine, and a small amount of ether which was given during the operation only. The vagi were cut and a tracheal cannula inserted. The animal inhaled through 7 per cent ammonia and exhaled through water. In fifteen minutes the respiration was stimulated enormously. There was also muscular twitchings increased reflexes and the animal required more ether. When 28 per cent ammonia was substituted the animal died in a few minutes in a typical ammonium tetanus.

*March 12, 1913.* To show the absorption of ammonia by the lungs. The ammonia in the blood of a dog was determined by Folin's method. The vagi were cut and the animal allowed to inhale ammonia through a tracheal cannula until twitching of the muscles began when the ammonia in the blood was again determined. Care was taken to avoid the ammonia in the room being a factor in the second determination. The normal blood contained less than 1 mgm. per 100 cc. This is taken higher than the true figure, which I find in a number of cases to be about 0.6 mgm.

After the inhalation, 10 mgm. per 100 cc. or more than ten times as much ammonia was found in the blood.

*April 1, 1913.* A dog was prepared as above and the ammonia in the normal blood estimated. After the animal had inhaled 7 per cent ammonia for twenty minutes the respiration was distinctly stimulated. The blood was again analyzed. The normal blood contained 0.6 mgm. per 100 cc. after twenty minutes inhalation the blood contained 1.6 mgm. or about three times as much. The large doses of morphine were given to avoid the need of ether during the experiment. Otherwise when the animal was stimulated by the ammonia it might be hard to decide whether it was the presence of the ammonia or the need of ether that was the cause of the muscular movements. In some cases where we found no stimulation of the respiration after inhalation of 7 per cent ammonia for twenty minutes, the dose of morphine was probably too large. Ammonium salts are also absorbed by the lungs.

*March 8, 1913.* A strong healthy dog about 9 kilos weight was given a large dose of morphine and a tracheal cannula inserted. Ether was given during the operation.

- 10.00 Poured 25 cc. 4 per cent ammonium chloride into the lungs and washed this in with 50 cc. of water.
- 10.05 Respiration much stimulated. Not asphyxia.
- 10.35 100 cc. 5 per cent ammonium chloride in saline was poured into the trachea. There was formed immediately, a dangerous amount of frothy fluid in the cannula so that the animal had to be raised to allow the fluid to flow out of the lungs. 195 cc. were removed by this procedure.
- 10.40 Distinct ammonium tetanus. Respiration also stimulated.
- 10.45 Convulsions continue.
- 10.50 Two grams of ammonium chloride in 25 cc. water poured into the lungs.
- 10.55 Animal died in a typical ammonia tetanus. The lungs contained fluid which was drained out. 105 cc. more fluid was found in the lungs than had been injected during the operation.

*March 13, 1913.* A dog, 8 kilos weight. Anaesthetized with morphine and ether. Tracheal cannula inserted and the vagi cut.

- 11.50 Two grams of ammonium chloride in 30 cc. saline were poured into the tracheal cannula. Respiration very slow.
- 11.55 Respiration stimulated.
- 12.00 Four grams more ammonium chloride in water were poured into the lungs. Reflexes elicited as easily as after strychnine.
- 12.05 Animal dies in an ammonium spasm. Blood at the commencement of the experiment contained less than 1.4 mgm. ammonium per 100 cc. Blood after the spasm contained 15.1 mgm. per 100 cc. or about twelve times as much.

#### THE EXCRETION OF AMMONIUM SALTS BY THE LUNGS

If the lungs be washed out with saline or water and the ammonium in the washings determined by the addition of sodium carbonate and distillation, it is found that normally there is only a trace present. If now ammonium salts be injected intravenously and the ammonium determined again after the experiment, it will be found that the amount of ammonium salt in the lungs is considerably increased. There is a probable source

of error here of which we wish to speak. When the lungs are washed out with saline before commencing the experiment it usually happens that there is a slight rupture as judged from a trace of blood in the second washing. We think the error very slight because normally there is only a trace of ammonia in the first washings as determined by the above method, and when we determine the normal amount in a number of animals, it is unnecessary to make the first washing in those we wish to use for the determination of ammonium excretion, and in cases where the first washout is omitted, the ammonia is found much increased after injection into the veins when there is not a trace of blood in the lung washings. Folin's method of ammonia determination gives similar results.

The following protocol will illustrate the differences in the ammonium content before and after ammonium injection.

A bull dog of about 15 kilos weight was anaesthetized with morphine and Grehant's mixture. Ether was used during the operation.

2.00 A tracheal cannula was inserted and 800 cc. of water poured into the lungs. Only 150 cc. was recovered. 30 cc. of saturated solution of sodium carbonate was added to this and 50 cc. distilled. Nessler's solution showed a trace of ammonia and titration required 0.05 cc.  $\frac{N}{10}$   $H_2SO_4$  to neutralize. Cochineal was used as the indicator.

2.30 till 3.15 7.5 grams of ammonium chloride in solution was injected into the jugular vein.

3.15 There was a considerable accumulation of frothy exudate in the cannula. The animal was elevated so that this could run out. 70 cc. were collected and 20 cc. sodium carbonate added. 20 cc. distillate required 3.8 cc.  $\frac{N}{10}$   $H_2SO_4$  to neutralize.

March 7, 1913. 70 cc. 5 per cent ammonium chloride was injected into the femoral vein of a 7-kilo dog in an hour. At the end of that time the lungs were washed out through a tracheal cannula with 0.9 per cent saline. 500 cc. was used and 400 cc. recovered. 50 cc. of saturated solution of sodium carbonate was added and a distillate of 50 cc. collected. And this required 3.1 cc.  $\frac{N}{10}$   $H_2SO_4$  to neutralize.

The washings in this case were absolutely free from blood. In every case there is a great increase in the ammonium content of the washings after the intravenous injection of ammonium chloride.

Instead of distilling off the ammonia Folin's method was used with similar results as the following protocol will show:

A 6-kilo dog was anaesthetized with morphine and ether. The lungs were washed with saline. 300 cc. were introduced of which 120 cc. was recovered. 100 cc. of this contained less ammonia than was neutralized by 0.1 cc.  $\frac{N}{50}$  acid. 50 cc. of 4 per cent  $\text{NH}_4\text{Cl}$  was injected into the femoral vein sufficiently slow to cause little disturbance of the heart or respiration. The animal soon commenced to twitch from the action of the ammonia and fluid gathered in the trachea. 38 cc. of clear fluid was recovered by elevating the animal and the ammonia in this determined. It contained sufficient to neutralize 10.5 cc.  $\frac{N}{50}$  acid or over 105 times that found in the normal animal before the injection of ammonium salt.

The experiments recorded, prove that ammonia and ammonium salts may be absorbed and excreted by the lungs, an opinion opposite to that held in the early part of the work.<sup>20</sup> Normally the amount of ammonia passing through the lungs must be extremely small. This, however, is not due to the impermeability of the lung tissue for the ammonium ion. That ammonia passes through the lung tissue has an important bearing on the explanation of the action of expectorants. Some of the action must be direct. Formaldehyde and some of its compounds pass through the lungs, the mechanism of the absorption of which must be different from the absorption of ammonium, and will be discussed in dealing with the former drug.

#### CONCLUSIONS

Traces of ammonia are excreted, and probably absorbed, normally by the lungs. Ammonia and ammonium salts in stronger concentration are readily absorbed and excreted. Absorption is shown by stimulation of the respiration, spasmodic twitching of the muscles and by an increase in the ammonium content of the blood. Excretion is shown by an increase in the ammonium content of the fluid of the lungs.

<sup>20</sup> McGuigan: The Journal of Biological Chemistry, 1912, xi, proceedings.

## SOME EXAMPLES OF THE EFFECT OF ASYMMETRIC NITROGEN ATOMS ON PHYSIOLOGICAL ACTIVITY

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The experiments of Cushny<sup>1</sup> and others have shown that optical isomers frequently have very different physiological activities. In most of the examples so far studied the isomerism has been due to the presence of an asymmetric carbon atom in the molecule. Hildebrandt,<sup>2</sup> however, records some examples of variations in activity due to asymmetric nitrogen atoms; working with quaternary coniine complexes he found that the  $\beta$  form was always more powerful than the  $\alpha$  variety. The differences in activity observed by him were small (about 1 to 2), and from his examples it might be concluded that the asymmetric nitrogen atom did not have such a profound influence on activity as an asymmetric carbon atom. I have recently had the opportunity of investigating some further examples of asymmetric nitrogen compounds, and find that the difference in activity may be large.

Jowett and Pyman<sup>3</sup> recently isolated from the bark of *Xanthoxylum brachyacanthum* the chloride of an alkaloid, which they identified as a methochloride of *l*-canadine. They further found that, of the two *l*-canadine methochlorides which are possible, owing to the asymmetry of the nitrogen atom (Voss and Gadamer),<sup>4</sup> the salt from *Xanthoxylum* corresponded to the  $\alpha$

<sup>1</sup> Cushny, Jour. of Phys., xxx p. 176, 1904; Cushny and Peebles, Journ. of Phys. xxxli, p. 501, 1905; Cushny, Journ. of Phys., xxxviii, p. 263, 1909.

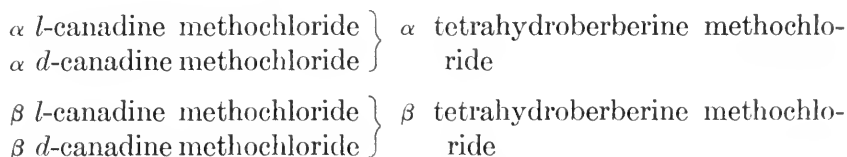
<sup>2</sup> Hildebrandt, Arch. f. exper. Path. u. Pharm., liii, p. 76, 1905.

<sup>3</sup> Jowett and Pyman, Trans. Chem. Soc., ciii, p. 290, 1913.

<sup>4</sup> Voss and Gadamer, Arch. d. Pharm., ccxlviii, p. 43, 1910.

variety. This appears to be the first recorded case of the isolation from a natural source of a substance presenting this type of asymmetry: and, since pharmacological activity has been attributed to several species of *Xanthoxylum*, it seemed of interest to make a quantitative comparison between some characteristic action of this methochloride and that of the isomeric  $\beta$  variety, which can be obtained synthetically.

Owing to the presence of an asymmetric carbon atom in canadine a dextro-canadine also exists and, therefore, two more isomers are possible, viz,  $\alpha$  and  $\beta$  dextro-canadine methochlorides. The ratio of activities of these was obtained indirectly through the  $\alpha$  and  $\beta$  tetrahydroberberine methochlorides, which are  $\alpha$  and  $\beta$  racemic canadine methochlorides. The possibilities are shown in the following scheme.



The four bodies actually compared were thus.

$\alpha$  *l*-canadine methochloride  
 $\beta$  *l*-canadine methochloride  
 $\alpha$  *ld*-canadine methochloride  
 $\beta$  *ld*-canadine methochloride

For the supply of these I am indebted to Dr. F. L. Pyman.

In common with other ammonium bases these alkaloids paralyse the motor endings in striped muscle, and it is this particular activity which has been studied; the other activities were not thoroughly investigated, and it is very probable that the ratios of activities obtained do not hold good for the other actions of these bases.

It was soon evident from a few preliminary experiments that the  $\beta$  salts were much more active than the  $\alpha$  salts. Thus if a series of frogs is injected with diminishing doses of  $\alpha$  and  $\beta$  canadine methochlorides, and the onset of "curare action" observed, a result like that in Table I is obtained, from which one



may say that  $\beta$  canadine methochloride is about ten times as active as the  $\alpha$  salt.

TABLE I  
*Frogs' average weight 20 grams*

$\beta$ l-CANADINE METHOCHLORIDE		$\alpha$ l-CANADINE METHOCHLORIDE	
Dose	Effect	Dose	Effect
<i>mgm.</i>		<i>mgm.</i>	
4.0	Complete paralysis in 7 min.	4.0	Complete paralysis in 30 min.
2.5	Complete paralysis in 8 min.	2.5	Nearly complete paralysis in 45 min.
1.0	Complete paralysis in 10 min.	1.0	Partial paralysis in 45 min.
0.25	Nearly complete paralysis in 50 min.		
0.10	Partial paralysis in 50 min.		

It appeared unlikely that an accurate comparison could be made by this method. Variations in individual frogs, apart from weight, and the difficulty in gauging the degree of paralysis, make comparisons difficult. More accurate comparison is obtained by the following method. The hind legs of a frog were perfused with Ringer's solution through the aorta just above the bifurcation into the iliac arteries. One sciatic nerve was stimulated at intervals of ten seconds, with single maximal induction shocks, and the height of contraction of the gastrocnemius muscle was recorded. The opposite limb was then excluded from circulation by a small clip on the iliac artery and Ringer's solution containing a known dilution of alkaloid was perfused through the stimulated limb. The height of contractions slowly diminishes as perfusion is continued and a curve of the onset of paralysis for a given dilution is thus obtained. Plain Ringer's solution is then substituted to study the rate of recovery. The other leg is then treated in a precisely similar manner, except that a known dilution of the other isomer is perfused through it. In this way a fairly accurate comparison of activities is obtained (see fig. 1 and 2.) The perfusion pressure was kept high in order to exclude effects of differences in perfusion rate and was, of course, the same for right and left legs.

Some experiments were performed by perfusing Ringer's solution through a paralysed or partially paralysed limb, until recovery was about complete; but it was found that, when once a muscle had been poisoned by one isomer, it never recovered completely within the rather narrow time limits of a perfusion experiment; so that it remained more susceptible to the same, or opposite isomer, and paralysis could be easily obtained with weaker dilutions than that originally needed. This method was, therefore, abandoned and a comparison of one limb, one isomer, against opposite limb, opposite isomer, was adopted.

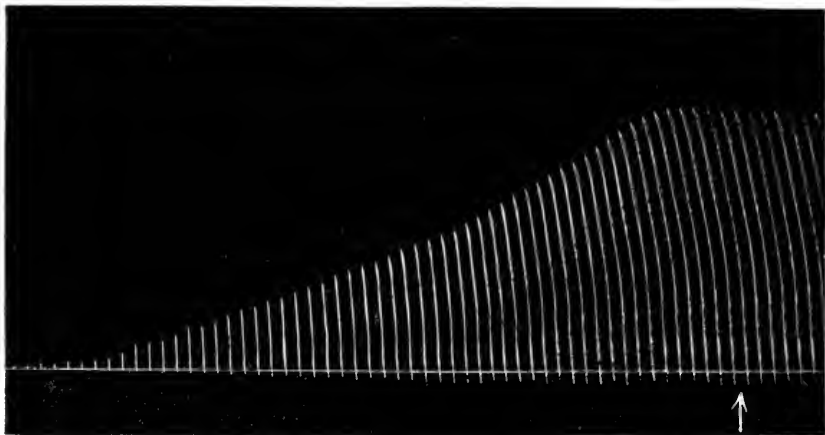


FIG. 1. EFFECT OF 1 IN 20,000  $\alpha$  TETRAHYDROBERBERINE METHOCHLORIDE

Figure 1 is a typical tracing of the effect of 1 in 20,000  $\alpha$  tetrahydroberberine methochloride; and figure 2 that of 1 in 20,000  $\beta$  tetrahydroberberine methochloride. It will be observed that the  $\alpha$  compound is much weaker than the  $\beta$ . By comparing weaker strengths of  $\beta$  tetrahydroberberine methochloride with 1 in 20,000  $\alpha$  on other frogs it was found that 1 in 80,000  $\beta$  produced approximately an equal effect.

Figure 3 is a chart representation of two experiments. The ordinates represent the height of gastrocnemius contractions, the abscissa time in minutes of perfusion with the drug. The records with 1 in 50,000  $\alpha$  racemic canadine methochloride and 1 in



FIG. 2. EFFECT OF 1 IN 20,000  $\beta$  TETRAHYDROBERBERINE METHOCHLORIDE

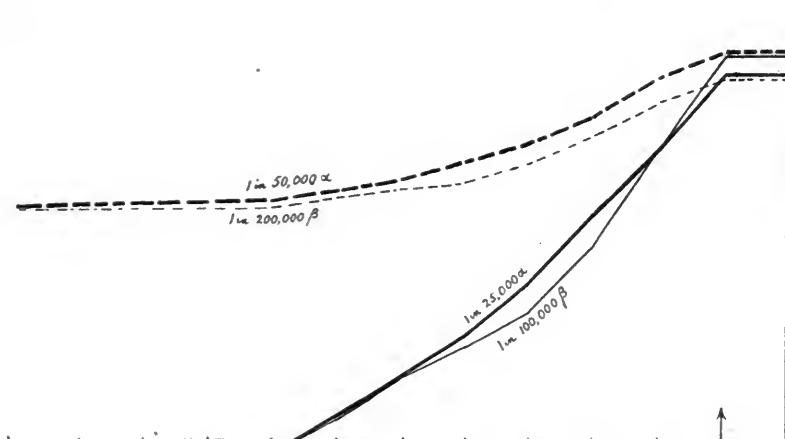


FIG. 3.  $\alpha$  AND  $\beta$  TETRAHYDROBERBERINE METHOCHLORIDES

200,000  $\beta$  racemic canadine methochloride (upper curves) show that, with these concentrations, the rate of onset of paralysis is very slow and not complete yet the two effects appear to be equal. The two lower curves are from another experiment where the concentrations of the alkaloids in the perfusion fluids were doubled, and it is evident that the curves are again closely similar. It may be concluded that  $\alpha$  racemic canadine methochloride is only one-fourth as powerful as the  $\beta$  form.

Figure 4 is another chart representation of two experiments with  $\alpha$  and  $\beta$  *l*-canadine methochlorides. In this case it was

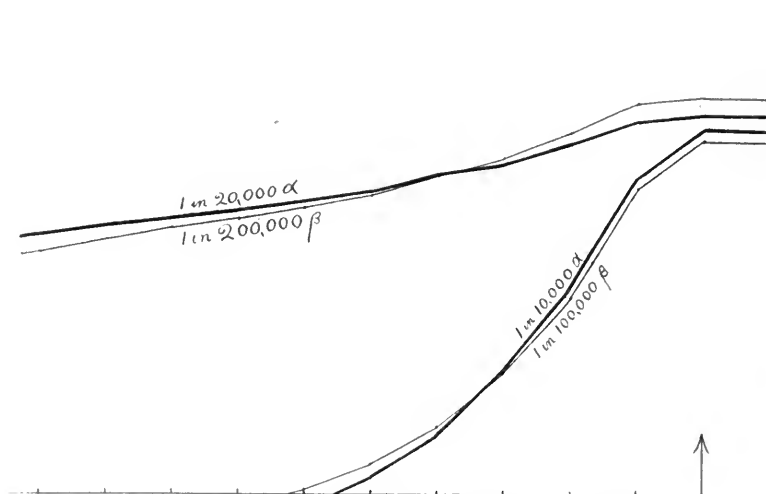


FIG. 4.  $\alpha$  AND  $\beta$  *l*-CANADINE METHOCHLORIDES

found that similar curves of paralysis were only obtained when the  $\beta$  *l*-canadine methochloride was perfused in at one-tenth the concentration of the  $\alpha$ . 1 in 10,000  $\alpha$  and 1 in 100,000  $\beta$  produce similar effects (lower curves). 1 in 20,000  $\alpha$  and 1 in 200,000  $\beta$  give smaller but equivalent effects (upper curves). It may be concluded that  $\alpha$  *l*-canadine methochloride is to  $\beta$  *l*-canadine methochloride as 1 is to 10. But in making up the solutions for these experiments crystalline salts were used and the  $\beta$  form crystallises with six molecules of water of crystallisation and the  $\alpha$  with only one. Correcting for this error, the true ratio is obtained of about 1 : 12 instead of 1 : 10.

In a precisely similar way the ratio of activity of  $\alpha$  *l*-canadine methochloride to  $\alpha$  *dl*-canadine methochloride was found to be 1 to 5.

The ratio of the activity of  $\alpha$  dextro-canadine methochloride to that of  $\beta$  dextro-canadine methochloride can easily be deduced from these data.

The previous experiments show that

$$(1) \alpha l : \beta l :: 1 : 12, \text{ or } 12 \alpha l = \beta l.$$

$$(2) \alpha l : (\frac{1}{2} \alpha l + \frac{1}{2} \alpha d) :: 1 : 5 \text{ or } 9 \alpha l = \alpha d.$$

$$(3) (\alpha l + \alpha d) : (\beta l + \beta d) :: 1 : 4, \text{ or } 4 \alpha l + 4 \alpha d = \beta l + \beta d.$$

Substituting  $\alpha l$  where possible in the last equation

$$4 \alpha l + 36 \alpha l = 12 \alpha l + \beta d$$

$$28 \alpha l = \beta d$$

$$\therefore \alpha d : \beta d :: 9 \alpha l : 28 \alpha l$$

$$\text{or } 1 : 3 \text{ approximately.}$$

The asymmetry of the nitrogen atom in the canadine methochlorides produces very different results in the two closely related *d*- and *l*-canadine methochlorides. In laevo-canadine methochloride the ratio is 1 : 12, in dextro-canadine methochloride it is 1 : 3. Further if the activity of  $\alpha$  laevo canadine methochloride be taken as unity the other isomers have values as follows.

$\alpha$  laevo canadine methochloride, 1

$\alpha$  dextro canadine methochloride, 9

$\beta$  laevo canadine methochloride, 12

$\beta$  dextro canadine methochloride, 28

At present no explanation of these various degrees of activity is forth-coming. The dextro canadine methochlorides have not been available in a pure state, and their properties must be included in any generalisation. It is worth noting, however, that  $\alpha$  laevo canadine methochloride is very easily soluble in cold water and that  $\beta$  laevo canadine methochloride is only sparingly soluble; also it crystallises with six molecules of water of crystallisation, as compared with one in the  $\alpha$  form. Some solubility differences might account for the different activities, but it must

be borne in mind that the solubility in water or saline is only one factor, and the solubility in the tissue of the myoneural junction is equally, if not more important. There does not seem to be any method available for testing the partition coefficient for these substances between circulating fluid and the junctional tissues. So that we may leave the speculation on one side until some method is devised for testing the theory.

#### ADDENDUM

##### *A simple apparatus for excluding make shocks*

In cases where stimulation at regular intervals is required, it is convenient to use some form of clock which makes and breaks at regular intervals the primary current of an induction coil. In this series of experiments the Palmer clock, which is designed to act as a time marker, was employed. The make shock was excluded from the stimulations by the following simple device.

A lever with unequal arms was mounted so that the short arm, carrying a piece of soft iron, could be drawn downwards by means of an electro magnet. The long arm was fitted with a light metal bar from which depended two platinum pins. These, in the position of rest, dip into two mercury pots insulated from each other. Each pot is connected with one terminal of the secondary coil used for stimulation, and the primary circuit includes battery, clock, electro magnet of apparatus and primary coil. At make of primary, the secondary coil is short circuited through mercury pots and platinum pins carried by the lever. It thus fails to stimulate. Almost immediately this short circuit is broken by the electro magnet raising the platinum pins out of the mercury cups, so that, on break of primary, the break induced shock passes through the stimulating electrodes before the platinum pins drop back into mercury cups, and so short circuit the secondary again (see diagram). The smooth working of the apparatus depends, apart from cleanness of all contacts, on the degree of dip of the platinum pins into the mercury. This must be sufficient to exclude all the make shock, and the pins must be

raised sufficiently high from the mercury to allow time for the break shock to pass before the secondary is short-circuited again.

It is evident that break shocks could be excluded, and make shocks employed to stimulate, by reversing the position of the mercury cups to the other end of the lever.

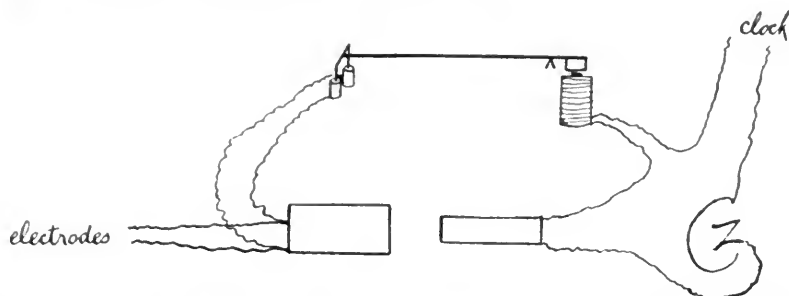


FIG. 5. DIAGRAM OF APPARATUS AND CONNECTIONS FOR OBTAINING SINGLE INDUCED BREAK SHOCKS BY MEANS OF CLOCK AND INDUCTION COIL

#### SUMMARY

1. An asymmetric nitrogen atom may exercise a profound influence on physiological activity.

2. In the case of the *l*-canadine methochlorides the  $\beta$  salt is 12 times as active as the  $\alpha$  isomer.

3. In the case of the *d*-canadine methochlorides the  $\beta$  variety is only about 3 times as active as the  $\alpha$  form.

4. In both cases the laevorotary base is weaker than the dextrorotary but not in equal degree.

5. No explanation of these variations in activity of the four isomers is satisfactory. It may possibly be connected with differences in solubility.

6. A simple apparatus is described for the exclusion of make shocks, when employing a clock and induction coil for single stimuli at regular time intervals.





# ON THE PHARMACOLOGICAL ACTION OF HELENIN, THE ACTIVE PRINCIPLE OF HELENIUM AUTUMNALE

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Helenium Autumnale has been known for a very long time. Barton in his *Flora of North America*<sup>1</sup> gives the following account of the plant, "Discordes describes *Ελενιον*, which Professor Martyn informs us was called in honor of Helen, consort of Menelaus, who cultivated a plant according to Herychius which destroyed serpents; according to other authorities, 'it sprang from her tears.'"<sup>2</sup> Gray says that it was named after Helenus the son of Priam.<sup>2</sup> Since then it has received a great many popular names, such as sneezeweed, sneezewort (the English sneezewort is not the same plant), swamp sunflower, false sunflower, yellow star, ox-eye, autumn sneezewort, and staggerweed. Its proper name according to Linnaeus<sup>3</sup> is *Helenium Autumnale*.

As early as 1635 there is a good botanical description given, together with a very excellent figure of the plant.<sup>4</sup> Gray in his *Synoptical Flora of North America*, gives probably the best description of the plant, naming twenty species.

It is a well known American plant found all over the United States, most abundantly from New York westward, and southward, and commonly grown for decorative purposes. It grows to a height of two or three feet, branching upwards, with large, showy, yellow flowers, blossoming in the autumn.

<sup>1</sup> Barton, Will P. C.: *A Flora of North America*. Philadelphia, 1821, p. 95.

<sup>2</sup> Gray Isa: *Gray's New Manual of Botany*, American Book Company, 1908 p. 844.

<sup>3</sup> Linnaei, Caroli: *Species Plantarum*, 1753, vol. ii, p. 886.

<sup>4</sup> Le Moyne S.: *Canadensum plantarum aliarumque nondum editarum historia*, Parisiis 1635, 4, page 238, prae. ind. tab.

As the name "Sneezeweed" implies, the plant has marked sternutatory properties. Inhalation of the powder, or dust of the dried plant, or even chewing a bit of the leaves, causes violent sneezing. This latter fact is noteworthy, as Helenin is non-volatile. The sneezing may possibly be accounted for by the fact that Helenin is a local irritant to the mucous membranes of the mouth, and in chewing the plant this local irritation may be strong enough to cause sneezing reflexly. I personally know of a dinner at a Yacht Club in Marblehead, where the room and tables were decorated with *Helenium Autumnale*. When the guests arrived, one after another began to sneeze and weep to such an extent, that all the flower decorations had to be removed. In 1633 in Gerarde's Herball there is an interesting article on a similar plant, the Sneezewort of Austria, in which the following description is given, "The herbe chewed and holden in the mouth, bringeth mightily from the braine, flimme flegme like Pellitore of Spaine. The juice mixed with vinegar and holden in the mouth, easeth much the pain of toothache."<sup>5</sup> As a therapeutic agent *Helenium Autumnale* has been advised by many as a tonic, snuff, febrifuge, and diaphoretic, and was used by the Winnebago Indians for "colds." A very similar variety, *Helenium Montana*, is described as, "Heads bitter and acrid. Hot as red pepper. Strong as whisky. Used for venereal diseases."<sup>6</sup> Bigelow in 1872 speaks of its use by medical practitioners, both in the United States, and in Germany, as a tonic, febrifuge, and errhine. He says, "It is known, and employed all over the country (U. S. A.), as a valuable errhine. The whole plant acts as such, but the flowers, especially the central florets, are the more powerful. It may be used in diseases of the head, deafness, amaurosis, headache, hemicrania, rheumatism, and congestion of the head and jaws. The shocks of sneezing are often useful in these cases when other remedies hardly avail."<sup>7</sup>

<sup>5</sup> Gerarde's Herball, London, 1633.

<sup>6</sup> U. S. Department of Agriculture, Contributions from the U. S. Nat. Herbarium, vol. vii, 1900-1902, p. 394.

<sup>7</sup> Bigelow, J. M.: Detroit Rev. Med. and Pharm., 1872, vol. vii, p. 199-201.

Kubo has lately described a substance, Grayanotoxin, the active principle of a Japanese plant, which causes the same local irritation of the mucous membranes as *Helenium Autumnale*, but its other pharmacological, and chemical properties, are quite different.<sup>8</sup>

*Helenium Autumnale* has a very marked poisonous effect on cattle, which at times eat it. As in some districts the plant is so abundant that it covers the ground for acres, it will be seen that it is quite an important and dangerous source of poisoning for cattle. Cattle living where it grows never touch it. But horses which have been for a long time under way, and have been long without food, on arriving in a district with whose vegetation they are not acquainted, are very apt to eat it with fatal results. It is said that cattle once having recovered from a case of poisoning, acquire a great craving for the plant. Dr. Phares gives an account of cases of poisoning in animals, which is quoted by Pammel in his *Manual of Poisonous Plants*.<sup>9</sup> The chief symptoms are respiratory difficulties, diarrhoea, and finally convulsions. Horses and mules succumb to the injurious effects of the plant quicker than other animals.

As an antidote which Dr. Phares has found effective, he mentions a pint or two of melted lard poured down the animal's throat. This in his experience has given very prompt and complete relief.

The above described poisonous properties of the plant seem to be characteristic of certain varieties only. *Helenium Montana* for instance, is reported to have no such action.<sup>10</sup>

The only variety from which an active principle has been isolated, as far as I have been able to ascertain, is the common *Helenium Autumnale*.

In 1910 E. Reeb<sup>11</sup> in Strassburg, isolated from the plant *Helenium Autumnale*, a crystalline substance, which had a very bitter

<sup>8</sup> Kubo, O.: Ueber Grayanotoxin, den giftigen Bestandteil der *Leucothoe Grayana* Max., *Archiv f. exp. Path. u. Pharm.*, 67, p. 110, 1912.

<sup>9</sup> Pammel, L. H.: *A Manual of Poisonous Plants*, Torch Press, Cedar Rapids, Iowa, 1911.

<sup>10</sup> The Stock Poisoning Plants of Montana. Bull. 26, U. S. Department of Agriculture.

<sup>11</sup> Reeb, E.: *Helenium Autumnale et son principe actif*, Mulhouse Imprimerie, J. Brinkmann 1910.

taste, and to which he gave the name Helenic Acid, and the formula  $(C_8H_{10}O_2)_n$ . This substance which had very marked sternutatory properties, and which caused lachrymation when he was working with the powder, Mr. Reeb sent to Professor Faust, asking that the pharmacological properties, might be more carefully studied. Professor Faust kindly furnished me with a number of preparations with which I have carried out the following experiments.

#### CHEMICAL DATA

As received from Mr. Reeb there were six crystalline preparations from various parts of the plant. His method of obtaining these preparations was in short as follows.

The part of the plant to be used was dried and powdered, and this was then extracted with chloroform. The extract evaporated to dryness, and the residue taken up with water. On filtering and allowing to stand, the Helenin falls out in crystalline flakes. In some cases this simple process was hindered by the presence of a dextrorotary sugar, in which case further extraction with toluol, etc., was necessary, before the Helenin could be crystallized out in a pure state.

In these six preparations there was no great difference in solubility and melting point; the production of sneezing and lachrymation, and the action on frogs seemed very similar. The entire amounts of the preparations, about 6 grams in all, were mixed dissolved in boiling benzol, after addition of animal charcoal, and recrystallized from this solvent. By this method very beautiful, long, white, needle-shaped crystals, showing the constant melting point  $167^\circ$  to  $168^\circ$  (uncorrected), were easily obtained.

Analysis of this substance showed it to be nitrogen free. C and H were determined by combustion, and O necessarily by difference.

#### I

Material used.....	0.1759	g.
H <sub>2</sub> O : 0.1202 g. of which H : 0.0133 g. :	7.64	%
CO <sub>2</sub> : 0.4446 g. of which C : 0.1212 g. :	68.93	%
By difference                      O : 0.0414 g. :	23.43	%

## II

Material used.....0.1263 g.

H<sub>2</sub>O : 0.0854 g. of which H : 0.0095 g. : 7.56 %

CO<sub>2</sub> : 0.3162 g. of which C : 0.0861 g. : 68.27 %

By difference O : 0.0307 g. : 24.17 %

The molecular weight by Beckman's method gave the following results.

Material used..... 0.2275 g.

Raising of the boiling point..... 0.104°

Benzol used.....20.00 cc.

From which the molecular weight 315, and the formula C<sub>20</sub>H<sub>22</sub>O<sub>3</sub>, was calculated, which corresponds with that obtained by Mr. Reeb.

	Calculated for C <sub>20</sub> H <sub>22</sub> O <sub>3</sub>	Found
C.....	69.56%	68.60%
H.....	7.24%	7.60%
Molecular weight.....	345	315

This substance crystallizes best and with great ease from benzol, being fairly soluble in hot benzol, while it is soluble with difficulty in the cold. With care beautiful, white, needle-shaped crystals, of 5 cm. or more in length can be obtained, which have a melting point of 167° to 168° (uncorrected). Helenin is very easily soluble in acetone, chloroform, glacial acetic acid, and 96 per cent alcohol. In ether and toluol it is soluble with difficulty, in petroleum ether, very insoluble; 100 cc. of water dissolve 0.3111 gram at 14.2°.

The reaction of the watery solution is neutral. It reduces alkaline copper mannite solution immediately on slight heating, but not after even long standing in the cold. Polariscopic examination of a saturated solution of Helenin shows no rotation, and a watery solution of Helenin, mixed with a little yeast, and incubated for twelve hours, gives no evolution of carbon dioxide gas.

A small quantity of Helenin was boiled for some time with a little dilute hydrochloric acid, and the solution divided into four parts. The first of these was made alkaline with sodium hydrate, a little copper mannite solution was added, and on slight heating a copious precipitate of red copper oxide immediately came down.

The second portion was neutralized with sodium carbonate, mixed with a little yeast, and incubated twelve hours. There was no evolution of carbon dioxide gas.

The third portion was examined with the polariscope. This showed no optical activity.

The fourth portion was treated with phenylhydrazine hydrochloride and sodium acetate. The boiled solution gave a brown smear but no crystals. Repeated attempts at purification of the amorphous substances obtained by decomposing Helenin with dilute, hot hydrochloric acid, with pyridine and with alcohol, both before and after boiling with animal charcoal, were equally unsuccessful.

The color reactions for carbohydrates, the Molisch and sulphuric acid tests were all negative. The digitalis reaction with solid potassium bromide and concentrated sulphuric acid, gives with a crystal of Helenin, a deep yellow color.

This inability on my part to isolate a crystalline osazone, the lack of carbon dioxide formation with yeast, the optical inactivity of Helenin solution after boiling with hydrochloric acid, and the negative results of the carbohydrate reactions, all point to the absence of a carbohydrate group in the Helenin molecule. Furthermore the fact that in the formula  $C_{20}H_{25}O_5$  there are five oxygen atoms only, would lead one to assume that this substance contains no carbohydrate group. If it were a glucoside, the highest sugar which in this case *could* be split off, would be a pentose.

A solution of Helenin in chloroform decolorizes a solution of bromine in chloroform completely, without the evolution of hydrobromic acid gas, on standing one or two minutes in the cold. The reduction of copper mannite solution by Helenin may be most probably explained by the *presence of double bonds in the molecule, i.e.,* Helenin is an unsaturated compound.

#### THE PHARMACOLOGICAL ACTION OF HELENIN

I have had none of the dried plant with which I could experiment, but in the literature one has abundant evidence of its local irritant action on the mucous membranes of the nose and eyes, causing sneezing and lachrymation. Dr. Phares<sup>12</sup> gives the

<sup>12</sup>The Stock Poisoning Plants of Montana. Bull. 26, U. S. Department of Agriculture.

only account which I could find of experimental poisoning in animals. Horses and mules, to which a decoction of the plant was given by mouth, are described as having diarrhoea, which would indicate a local irritant action on the gastrointestinal tract. Very marked respiratory disturbances are also described, and in sheep epileptiform convulsions.

The crystalline Helenin has a very bitter taste. The local action is most marked on mucous membranes. When working with it in powdered form, it causes violent sneezing, and lachrymation. A little of the powder, blown into the noses of dogs, and rabbits, causes sneezing and local irritation, but the action is not as marked as in human beings, and soon passes off. A saturated watery solution, dropped into the eye of a rabbit, causes considerable lachrymation and evident irritation, as shown by the way in which the rabbit rubs the eye. These symptoms pass off in a few minutes and no other changes are noticeable for ten or twelve hours, after which time there is no injection, but considerable swelling of the lid. This increases for the next forty-eight hours and then passes off, leaving the eye apparently normal. I applied Helenin in the form of a salve on my upper arm, and it caused no redness or irritation, I also injected a half saturated watery solution of the same under the skin. This caused a burning sensation lasting some ten minutes, and was followed by considerable oedema over an area of 4 or 5 sq. cm., lasting two or three days, and not entirely disappearing for two or three weeks. There was however no hyperaemia, and no sign of abscess formation. Injection of the saturated watery solution in doses of 0.003 to 0.006 gram subcutaneously in cats and dogs, showed the same local effects, evidently painful, as the animal moves carefully and guards the point of injection. In all cases localized oedema followed, but without inflammation or abscess formation. No general or abortive action was shown after subcutaneous injection.

#### ACTION ON THE INTESTINAL TRACT

For the mucous membranes of the intestinal tract Helenin is evidently a severe irritant. its action here being analagous and

directly comparable to that on the eye, mucous membranes of the nose etc., but much more marked. When applied to the nose, sneezing follows; thus removal of the active substance. In the digestive tract, (intestine) speedy removal is impossible, hence the severe symptoms. 0.195 gram of the crystalline Helenin were dissolved in water, and given to a dog weighing 7100 grams by the stomach tube. This caused vomiting of a watery fluid in five minutes, followed by severe sneezing. Three minutes later there was marked tenesmus followed by first solid and then liquid stools. The dog then remained quiet for some time, after which he vomited very often, with constant retching and tenesmus, the vomitus being at first yellowish and slimy, later bloody. The next morning the dog was found dead.

Autopsy showed the stomach and upper intestine filled with dark bloody fluid. The lower gut contained reddish colored, half formed faeces. The stomach wall and the upper three quarters of the intestine showed swollen papillae and very marked injection. The lower quarter was only slightly injected. The other organs showed nothing abnormal except the kidneys, which were slightly swollen and reddened.

The examination of the urine gave the following results. A large amount of albumin was present. There were many casts in the sediment, chiefly granular, epithelial and hyaline. There were also numerous red blood corpuscles, and a few leucocytes, besides many spermatozoa.

Two other dogs were given slightly smaller doses of Helenin per os, 0.180 gram. They both vomited in the same manner, and sneezed severely immediately after vomiting. They were then quiet for some time, after which vomiting and retching again set in. One of these dogs was used as a control, and given no antidote. He had considerable vomiting throughout the day accompanied by loose movements of the bowels, which lasted for several days. Appetite also was very poor, the dog eating nothing for several days. After this he gradually recovered completely.

The second dog was given in the midst of his severe attacks of vomiting, 50 cc. of sweet almond oil by the stomach tube.



In ten minutes he became apparently perfectly normal again, and set up a loud barking at the rabbits in the next cage.

The same dose (0.195 gram) given to a 2150 gram rabbit by the stomach tube caused no symptoms except a slight loss of appetite. The stools at no time gave a guajac test for blood. The examination of the urine was negative.

#### GENERAL RESORPTIVE ACTION

In frogs, watery solutions of various amounts were given in the dorsal lymph sac. After fairly large doses, (0.003 gram) the respiration was noticed to become more frequent, and deeper. There was a slight increase in the response to mechanical stimulation, respiration shallow and slow; oedema of the back, belly and legs was distinctly noticeable. Death usually occurred within five or six hours after doses of 0.003 gram. The minimum lethal dose, which caused death, after five days, was found to be, for a twenty-five gram *Rana esculenta*, 0.0009 gram. Post mortem examination showed in all cases serous watery oedema of the dorsal and abdominal lymph sacs and legs, 25 gram frogs gaining 10 to 14 grams in weight. On opening the abdomen, the lungs were found extending to the pelvis. Heart pale and partially contracted. Stomach and intestines very slightly injected, otherwise normal.

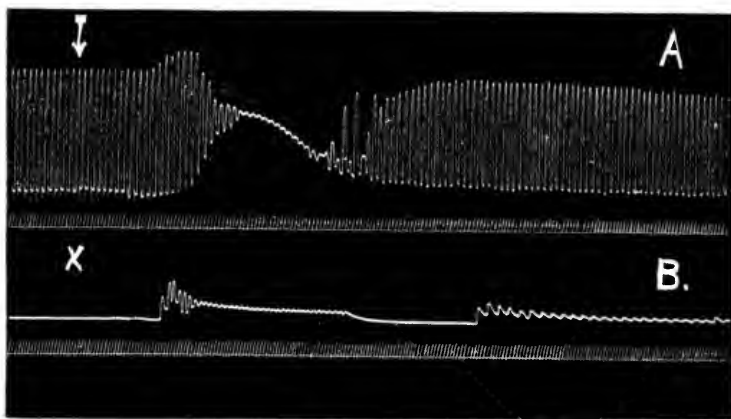
Intravenous injections of 0.0018 to 0.009 gram per kilo into rabbits, causes during the time of injection, a short respiratory standstill, followed immediately by great unrest, violent respiration and general collapse, the rabbit lying on its side and panting. After a few seconds the respiration becomes more quiet, but still continues forced and rapid. These symptoms gradually subside, and the rabbit gets up and moves about as usual. In two deaths which occurred, both with doses within these limits, autopsy showed on macroscopic examination, nothing abnormal. The heart was found stopped in systole.

Intravenous injection of 0.003 gram per kilo in a dog caused at first respiratory difficulties similar to those in the rabbits. The dog then appeared somewhat sad. Remained very quiet, and

after an hour vomited a little, and then gradually recovered. The stools showed no blood by the guajac reaction. Urine examined during the next six days was normal.

#### ACTION ON THE CIRCULATION

Injection of 0.002 to 0.004 gram of the watery solution of Helenin into the dorsal lymph sac of frogs (*temporaria*), causes a gradual paralysis of the heart. At first the systole remains good, the diastole being slightly prolonged and fuller. Rate not markedly changed, no irregularity in the beat. Gradually the diastole

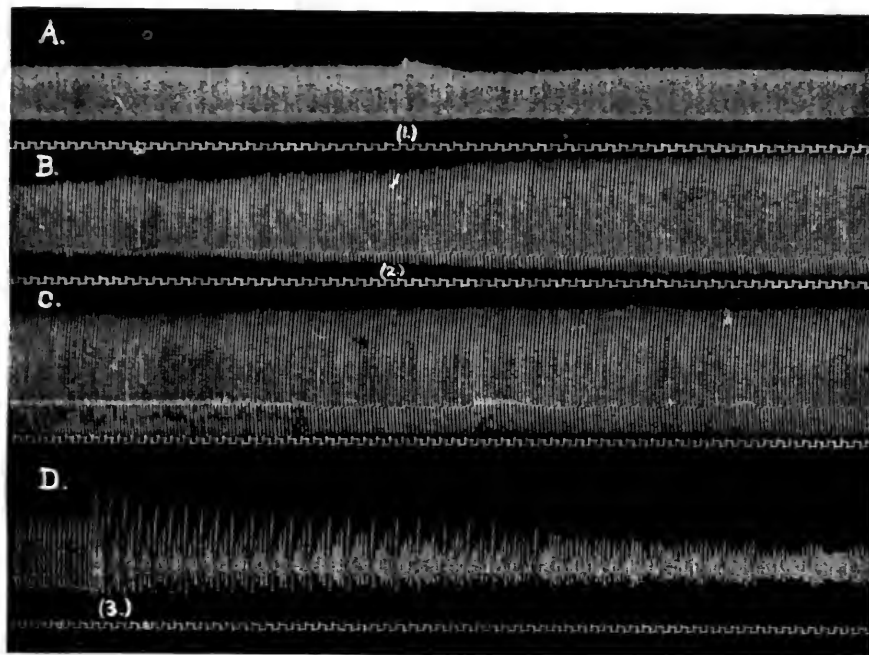


CURVE I. SUSPENDED FROG'S HEART. ENGELMAN'S METHOD

At X, Curve A, 0.0018 grams Helenin added to the 1 cc. of perfusion liquid. Curve B, same heart twenty minutes later. Time in seconds.

becomes longer and fuller, the systole is weakened, and the heart finally comes to a standstill in one or two hours in a semi-dilated condition. The same is noticed after direct application of the watery solution to the heart muscle, only the action is quicker. This result is in no way influenced by atropin, so that one may conclude that the action is a direct one on the heart muscle. Tracings of the isolated frog's heart gave similar results, both when the drug was added to the perfusion liquid and when the heart was perfused with the Ringer and gum arabic solution, and the drug placed in the liquid surrounding the heart.

With the excised and suspended heart by Engelman's method, where the drug was brought directly into the heart cavities in doses of 0.0018 gram in 1 cc. of salt solution, a sudden marked systolic contraction, followed by twenty or thirty very small beats occurred. Then there was a sudden increase in the size of the beat to normal, lasting a short time, after which paralysis set in as with the heart in situ (fig. I).



CURVE II

## ISOLATED RABBIT'S HEART. LANGENDORFF'S METHOD

Weight of Rabbit = 1730 grams. Weight of Heart = 6 grams. Perfusion liquid = 300 cc. of equal parts 0.85 per cent salt solution and defibrinated rabbit's blood. Temperature of liquid =  $37.6^{\circ}$  to  $37.8^{\circ}$ . Pressure = 65 mm. Time in one second intervals.

Curve A. = normal. At (1) heart perfused with 5 mg. Helenin per 100 cc. of perfusion liquid at 6.19 p.m.

Curve B. Same heart. At (2) perfused with normal perfusion liquid. 6.24 p.m.

Curve C. Same heart perfused with 5 mg. of Helenin per 100 cc. of perfusion liquid at 6.30 p.m.

Curve D. Same heart at 6.40 p.m. At (3) = 7.00 p.m.

In the isolated mammalian heart by Langendorff's method, the curves were very similar (fig. II). Here doses of 0.060 gram in 300 cc. of perfusion liquid (one-half defibrinated blood, and one-half salt solution 0.85 per cent) in a rabbit's heart weighing 6 grams caused at first more powerful, greater and slower contractions, lasting twenty minutes, after which a gradual weakening of the systole, a slowing of the rate and arrhythmia set in, standstill occurring in twenty-four minutes after beginning the perfusion of the heart with Helenin.

Blood pressure experiments on curarized rabbits, with artificial respiration, show after injections of 0.007 to 0.014 gram per kilo, a sudden rise of 10 to 20 mm. followed by a gradual fall to the same degree below normal, with a gradual return to normal. The size of the individual pulse beats was increased, and the rate slowed, which corresponds to the results of experiments on isolated hearts of frogs and rabbits. The slight variation in the blood pressure may be accounted for as a secondary action due to the effect of the drug on the heart muscle, and on the respiratory centre, both of which are considerably affected. This is shown in figures III and IV, in which the respiration is written by means of a Marey tambour, connected with a cannula placed in one nostril of a rabbit, the lower curve being the blood pressure, written by means of a mercury manometer connected with a cannula tied into the carotid artery.

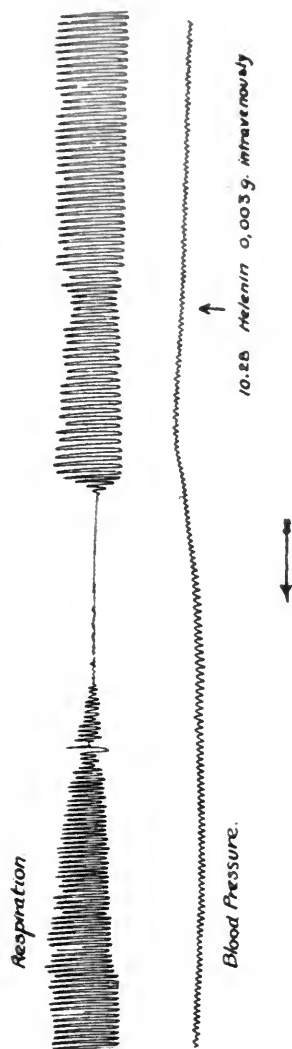
#### THE ACTION ON THE RESPIRATION

The action on the respiration is shown by a study of the same curve, and was also determined by the measurement of the actual amount of air expired, the results of which latter experiments are plotted in figure V. The ordinates give the volume of expired air, the number, and average volume of each respiration respectively, taken during thirty second intervals, time being the abscissa. It will be seen here that the volume of expired air is greatly decreased after each injection, with a gradual return toward, but not quite, to normal. The rate on the other hand is proportionally increased, and the volume of each respiration is proportionally decreased.

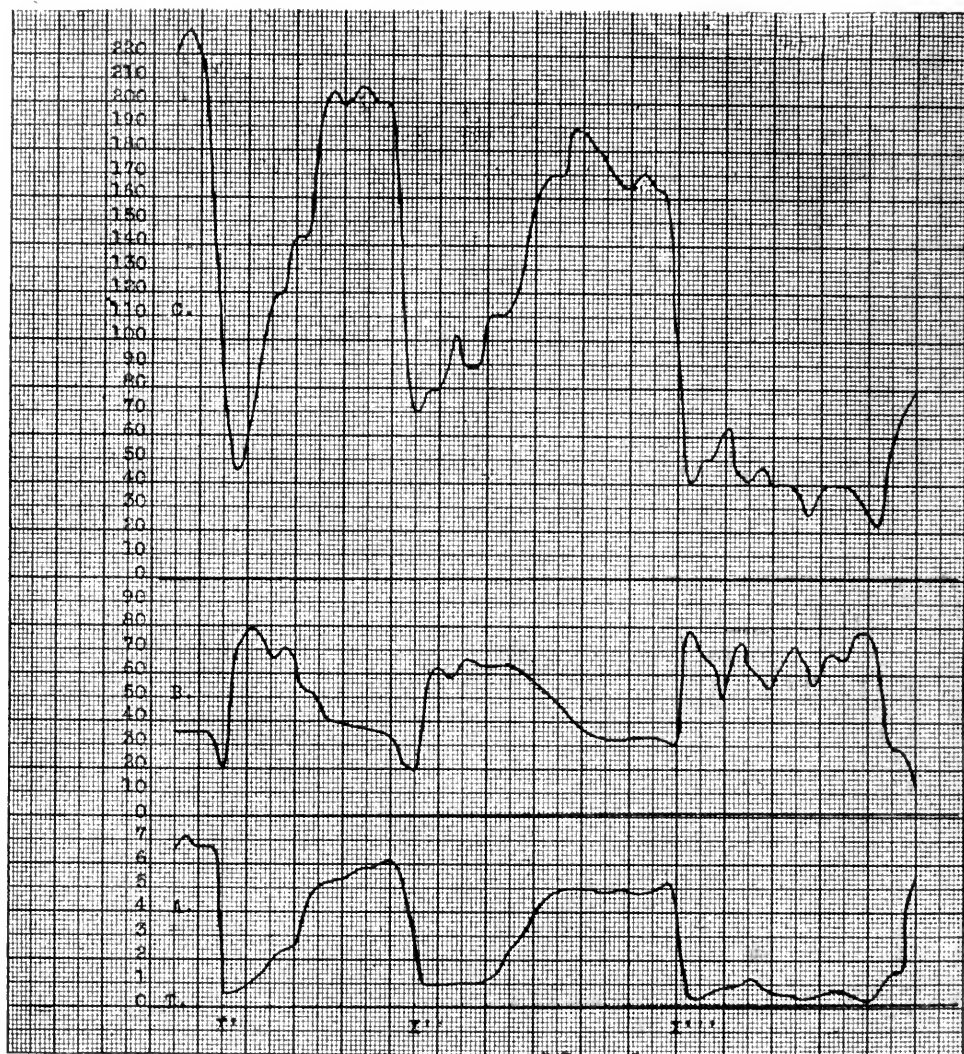


CURVE III. RABBIT 1950 GRAMS. RESPIRATION CURVE WRITTEN BY MEANS OF A MAREY TAMBOUR, CONNECTED WITH A CANNULA IN ONE NOSTRIL OF THE RABBIT

Blood pressure curve written by means of a mercury manometer; cannula tied into one carotid.



CURVE IV. A LATER INJECTION OF HELENIN IN THE SAME EXPERIMENT AS IN CURVE III



CURVE V

A, average volume of each respiration; B, number of respirations in each thirty seconds; C, volume of expired air in each thirty seconds; T, time in minutes: x', x'', x''', intravenous injections of 6.0 mg., 6.0 mg., 15.0 mg. of Helenin.

These curves thus confirm the observations on animals after intravenous injections, in which the respiration first stopped, then increased in force and frequency, and gradually returned to normal, or came to a gradual standstill, according to the amount injected.

#### ACTION ON THE RED BLOOD CORPUSCLES

Helenin solutions in normal physiological salt solutions, in concentrations varying from a mere trace of Helenin, to a saturated solution show no haemolytic action on washed rabbit's corpuscles, after standing for two hours in the thermostat.

#### ACTION ON STRIPED MUSCLE

Curves taken from a suspended gastrocnemius muscle of a frog, stimulated at regular intervals, show a slight increase in contraction of the muscle, followed by gradual paralysis, the action being very similar to that seen in the heart muscle. Teased muscle preparations in one-half saturated Helenin solutions, show no sudden contraction of the muscle fibers, but a slight opacity. Helenin has therefore a slight action on the muscle itself which might lead to the conclusion, that it is a protoplasmic poison. To decide this question, watery solutions of Helenin were allowed to act on the ciliated epithelium of the frog's oesophagus, and on Amoebae, Vorticelli, Paramaecia, and hay bacilli. Ciliary movements were not affected by weak or saturated solutions for over an hour. The Amoebae and Vorticelli were not affected for fifteen minutes, after the addition of a saturated Helenin solution, later they became only sluggishly active. After an hour practically all movements had ceased in the Amoebae and Vorticelli. The Paramaecia and hay bacilli were less affected. This slight action on protoplasm might be due to the difficulty with which the Helenin is absorbed by these cells.

On account of the widespread use of the plant against fever, its action on the body temperature after a "Fieberstich" was studied. Under aseptic precautions a "Fieberstich" was done on each of two rabbits, weighing 2400 grams and 2600 grams respectively. The temperature rose in the first from 39.2° to 40° and in the



CURVE VI. CURVES MADE BY STIMULATION OF A SUSPENDED GASTROCNEMIUS MUSCLE OF A FROG, STIMULATED AT REGULAR INTERVALS BY THE SECONDARY CURRENT OF AN INDUCTION COIL

The upper curve is normal. The lower is made while painting the muscle with a saturated solution of Helenin in 0.7 per cent NaCl.



other from 39.1° to 40.9° in five hours and thirty minutes. Each was then given 0.015 gram Helenin in an ear vein. Temperature taken at intervals for seventeen hours showed no fall below forty degrees in either. One rabbit underwent an abortion. It would appear from these results, that Helenin has no antipyretic action comparable to that of morphine or the bodies of the antipyrine group.

#### SUMMARY

Helenin, the active principle of *Helenium Autumnale*, is a crystalline substance of the empirical formula,  $C_{20}H_{25}O_5$ ; its melting point is 167° to 168°, and in water solution it reacts neutral. It reduces alkaline copper mannite solution on slight warming, but does not reduce this solution even on long standing in the cold. It gives with yeast, both before and after boiling with dilute hydrochloric acid solutions, no carbon dioxide gas formation, is optically inactive, and gives no osazone. The color test for carbohydrates with the Molisch reaction is negative. It contains then no carbohydrate group in the molecule, and is therefore no glucoside. It decolorizes solutions of Bromine in chloroform after one or two minutes in the cold completely, without evolution of hydrobromic acid gas. Accordingly one can conclude that Helenin contains unsaturated bonds, double linkages, which probably account for its property of reducing copper mannite solutions.

The pharmacological action of crystalline Helenin corresponds essentially with that of the entire plant, *Helenium Autumnale*. It is a very active local irritant to the mucous membranes, causing sneezing and lachrymation, when the powdered substance comes in contact with the mucous membranes of the nose and eye, gastric and intestinal irritation with vomiting and diarrhoea, when taken by mouth and pain and oedema when injected under the skin. In cases of lethal poisoning acute gastroenteritis is the cause of death, when given per os; failure of heart when injected intravenously.

Helenin causes a gradual paralysis of the heart, the action being exerted on the heart muscle itself. On the blood pressure

it has little effect, except secondarily. On the respiration the action is very severe. The rate and force of respiration being increased, but the amount of expired air decreased.

On the kidney the amounts injected in these experiments appear to have only a slight effect.

On skeletal muscle it causes a rapid decrease of contractility, and in teased muscle preparations it causes a slight opacity of the fibers.

Helenin can not be classed under the protoplasmic poisons and has no noticeable action as an antipyretic.

Both very dilute and saturated solutions of Helenin in physiological salt solution, have no haemolytic action on the washed red blood corpuscles of rabbits.

The pure crystalline Helenin produces the same symptoms when given to animals, as those described many years ago in cases of poisoning in cattle after ingestion of the plant. The giving of melted lard to cattle poisoned by the plant would appear rational from the results of my experiment with the active principle Helenin, in which oil was given internally to a dog per os. This treatment markedly decreased the local irritant action on the intestinal tract.

It seems very probable that this plant may be a cause of fall-hay fever.

Concerning the many medicinal uses of Helenin mentioned in the literature, as a tonic, snuff, febrifuge, anthelmintic, diaphoretic, and as an antidote for "colds" and venereal diseases, its pharmacological properties most certainly justify its use as an errhine. It could be used as a stomachic in small doses on account of its bitter taste and its irritant action on the mucous membranes of the stomach.

As a vermifuge its intense local irritant action on the mucous membrane of the stomach would exclude its use except in very dilute solutions, under which conditions its action on the parasites would be doubtful. The difficulty of its resorption from the intestinal tract would on the other hand, be a favorable property for this purpose, if an action on intestinal parasites could be demonstrated.

The pharmacological properties of Helenin, do not justify its use as an antipyretic, or in any of the other above mentioned diseases.

I wish here to express my thanks and appreciation to Prof. Edwin S. Faust for assistance rendered me in carrying out this work.



# A STUDY OF THE ACTION OF VARIOUS DIURETICS IN URANIUM NEPHRITIS, WITH SPECIAL REF- ERENCE TO THE PART PLAYED BY THE ANES- THETIC IN DETERMINING THE EFFICIENCY OF THE DIURETIC<sup>1</sup>

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*From the Laboratory of Pharmacology of the University of North Carolina*

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In a previous number of this journal (1), a report was made on the action of various diuretic substances in animals rendered nephritic by uranium nitrate. The animals employed in this series of experiments could be classified, on a basis of their response to diuretics, into three groups: an anuric, a practically anuric, and a diuretic group.

<sup>1</sup> Presented in abstract before the Society for Pharmacology and Experimental Therapeutics, Cleveland, December 30, 1912. Aided by a grant from the fund for Scientific Research of the American Medical Association.

In the present investigation, which is a direct continuation of the previously mentioned experiments, the same general grouping of the animals can be established. It has been experimentally demonstrated, however, that the practically anuric group is an unnecessary refinement in the classification, for the condition of partial anuria should be interpreted as a forerunner of the anuric state which sooner or later develops, provided the experiment be continued. Therefore, in the present report the observations will be confined to two groups of nephritic animals: a diuretic group and an anuric group.

The present investigation shows, also, that whether or not the animals are diuretic or anuric following the anesthetic depends very largely upon the anesthetic employed and upon the age of the animal. As a result of this observation, in addition to a continuation of the study of the response of the pathological kidney to diuretics, it becomes especially necessary to consider the response of the pathological kidney to different anesthetics.

#### REVIEW OF PREVIOUS EXPERIMENTS

In the experimental work previously published it was shown that when dogs were given uranium nitrate subcutaneously in the dose of from 5 to 10 mgs. the animals developed an albuminuria, which was followed within twelve to forty-eight hours by a glycosuria. The output of sugar in the twenty-four hour specimen of urine varied within wide limits: 0.25 to 3.22 per cent. No determination of acetone or acetone bodies was made.

It was also shown that either just prior to the development of the albuminuria, or associated with its development the output of urine increased, and that with the development of the glycosuria the animal became highly polyuric. For instance, in experiment 8, of the previously mentioned series the animal was receiving 500 cc. of water daily; the average output of urine prior to the uranium was 513 cc.; while following the uranium and with the development of a nephritis and a glycosuria the urine increased to 1310 cc.

The microscopic examination of the urine constantly showed the presence of erythrocytes, usually few in number, and tube

casts. Early in the nephritis, with the first appearance of albumin, the hyaline cast predominated, but later when the albuminuria had become more marked, granular casts predominated over the hyaline type.

When such animals which were nephritic, glycosuric, and polyuric were anesthetized by either Gréhan's anesthetic or by morphine-ether, they grouped themselves so far as their response to diuretics was concerned, into two main groups: an anuric group and a diuretic group.

The physiological study of these two types of animals with special reference to their response to diuretics, showed, in the first place, that no change had been induced in the height of general blood pressure to account for the difference in the output of urine in the two groups. Secondly, oncometric determinations of the local renal circulation showed that the response of the renal vessels in both groups was either normal or hyperactive. This was true for substances such as caffeine and theobromine which are supposed to influence the renal circulation principally through a local vascular effect, as well as for such a substance as digitalin, whose local effect on the blood supply of the kidney is induced through its general effect upon the circulation.

As a result of the above mentioned study of the general and local vascular response of the nephritic animals, the cause of the stoppage of the urine flow in the anuric group was thought not to be due to any vascular change.

The histological study of the kidneys from the diuretic and from the anuric groups showed a marked difference in the degree of epithelial involvement, whereas the vascular pathology in the two groups was practically identical.

In those animals that remained diuretic following the anesthetic, the epithelial involvement was slight or absent. The cells were not only not increased in size, but frequently they showed an undoubted shrinkage with an associated increase in the size of the lumen of the tubules. The cells stained well, the nucleus was not pyknotic, and the cytoplasm of the cells showed but slight or no vacuolation.

On the other hand, in those animals which became anuric following the anesthetic the epithelium of the tubules of the labyrinth, and especially of the proximal convoluted tubules, showed an acute swelling, which was remarkable for the rapidity with which it developed. As a result of the swelling, the lumen of the tubules was either greatly encroached upon or completely occluded. In some animals the swelling was so acute that the cells had not had time to undergo any marked degenerative change, while in other animals the epithelium was severely vacuolated, staining was imperfect, the nuclei pyknotic, and the cytoplasm in various stages of necrosis.

As a result of these observations, I was inclined to the belief that in a uranium nephritis the epithelial changes were more responsible for the reduction in the output of urine than were the vascular changes.

The continuation of these experiments, which will now be reported, serves in great measure to confirm this belief, and also brings into consideration the part played by different anesthetics and by the age of the animals in precipitating these epithelial changes.

#### DISCUSSION OF THE TECHNIQUE EMPLOYED IN THE EXPERIMENTS

The operative technique employed in the following experiments has been identical in every particular with that employed in the experiments of the previous investigation. There have, however, been made, for the sake of accuracy, slight changes in the quantity of the nephrotoxic substance employed; and also additional diuretic fluids have been used. In place of giving from 5 to 10 mgs. of uranium nitrate to the animals without regard to their weight, there was a determination made of the dose of uranium which was competent to induce a nephritis and a glycosuria without rendering the animals in the least toxic and without inducing gastro-enteric changes which are usually manifested by vomiting and a diarrhoea.

The dose of uranium nitrate when given subcutaneously in the dog which is sufficient to induce the desired changes in the kidney without the undesirable gastro-intestinal complications



has been found to be 6.7 mgs. per kilogram. This is the dose of uranium which has been constantly employed in all of the experiments.

Only animals in apparently perfect health were selected for the experiments. They were placed in metabolism cages and given daily by a stomach tube a known and constant quantity of water. The diet consisted of bread and uncooked meat.

The urine was collected at 4.30 p.m. each day and examined qualitatively for albumen, sugar, and acetone, and quantitatively for sugar. In making both the qualitative and quantitative sugar determinations, Benedict's (2) reagents were uniformly employed, for the reason that these solutions allow of more delicate determinations and do not deteriorate upon standing. No preservative was used in the urine.

These observations were made for three days prior to the administration of the uranium. On the third day, the first uranium injection was made and repeated at the same time on the fourth day, the experiment being performed on the fifth day that the animal had been under observation. Such a routine is necessary, for the changes in the urine, especially the output of glucose, are influenced to some extent by the time which has elapsed following the uranium injections.

As result of the foregoing study of the urine the use of an animal with a naturally acquired nephritis was excluded. None of the animals prior to the use of the uranium showed the presence in the urine of either glucose or acetone.

The diuretic substances which have been employed in these experiments include those which were used with the first series of animals; and, in addition, various salts which were used in solutions isotonic with 0.9 per cent and 2 per cent sodium chloride.

The osmotic pressure of some of the salts which have been employed has never been accurately measured. This, therefore, introduces an element of error into the calculations, which were conducted in order to obtain solutions of these salts isotonic with 0.9 per cent and 2 per cent sodium chloride. The calculations were made for solutions at 37.5°C. and at this temperature the solutions were introduced into the animals. For these

calculations, I am indebted to Dr. J. E. Mills, of the University of South Carolina.

The following solutions have been employed in the study of the response of the nephritic kidney to diuretic substances:

Caffeine.....	1 to 2 of a 1 per cent solution per kilogram
Theobromine.....	1 to 2 of a 1 per cent solution per kilogram
Digitalin.....	0.5 to 1 mg. per kilogram
Sodium chloride solution, 0.9 per cent.....	5 to 10 cc. per kilogram
Sodium chloride solution, 2 per cent.....	5 to 10 cc. per kilogram
Sodium carbonate, 0.9 per cent.....	5 to 10 cc. per kilogram
Sodium carbonate, 2 per cent.....	5 to 10 cc. per kilogram
Sodium sulphate, 0.9 per cent.....	5 to 10 cc. per kilogram
Lithium chloride, 0.9 per cent.....	5 to 10 cc. per kilogram

#### THE EFFECT OF URANIUM NITRATE ON THE OUTPUT AND COMPOSITION OF THE URINE IN YOUNG AND IN FULL GROWN ANIMALS

As has been previously stated, the present series of experiments serve not only for a study of the efficiency of various diuretic substances, but they also serve to demonstrate the difference in the response of animals of different ages to the same quantity of a toxic substance, such as uranium. The differences here referred to are those which are manifested in the total output of urine and in the composition of the urine.

During the course of this paper the animals referred to as "young," are animals not over one and a half years old; the "adult" animals are those varying in age between one and a half and six years; while the old animals are certainly over six years old. The classification is, of course, a more or less arbitrary one, though it is based to some extent upon a knowledge of the average life of the dog.

Falling in the group of young animals are eight dogs. Four of these animals were from the same litter, and at the time of the experiments were between four and a half and five months old. Two other animals were three months and three weeks old; while the remaining two dogs, young adults, as accurately as could be ascertained, were about one year and two months old.

The animals were given daily a known and constant quantity of water and allowed a mixed diet of bread and raw meat. Following the preliminary period of observation of three days, they were given subcutaneously on two successive days 6.7 mgs. of uranium nitrate per kilogram. The two young adult animals received 500 cc. of water daily, while the other members of the group received 400 cc.

On the second day of the uranium injections these animals showed the following changes in output and in composition of the urine:

As will be seen from table 1, these animals, following the development of a glycosuria, became polyuric. The output of glucose, especially by the puppies, is remarkably constant for the different animals, while the presence of acetone varies. The

TABLE 1

AGE	WATER	URINE	ALBUMEN	SUGAR	ACETONE
	cc.	cc.		per cent	
Young adult.....	500	830	Present	1.06	Trace
Young adult.....	500	1015	Present	0.701	Trace
Puppy.....	400	790	Present	0.202	None
Puppy.....	400	780	Present	0.446	None
Puppy.....	400	740	Present	0.077	None
Puppy.....	400	605	Present	0.35	Trace
Puppy.....	400	910	Present	0.434	Trace
Puppy.....	400	910	Present	0.86	Trace

quantity of acetone in the urine of these young animals is exceedingly small and its detection would frequently have been missed without a microscopic examination of the tested distillate for the presence of crystals of iodoform.

The group of animals referred to as "adult" animals were kept on the same diet as the younger animals, and like the younger animals were given 6.7 mgs. of uranium nitrate per kilogram on two successive days. The changes in the output of urine and in the composition of the urine are indicated in table 2, which represents the course of eight characteristic experiments following the uranium injections and prior to the use of an anesthetic.

TABLE 2

AGE	WATER	URINE	ALBUMEN	SUGAR	ACETONE
	<i>cc.</i>	<i>cc.</i>		<i>per cent</i>	
Adult.....	500	1240	Abundant	2.17	Pronounced
Adult.....	300	645	Abundant	2.56	Pronounced
Adult.....	500	980	Present	2.17	Present
Adult.....	500	1180	Present	3.625	Present
Adult.....	500	1025	Present	2.08	Present
Adult.....	500	1515	Present	2.08	Present
Adult.....	500	1130	Present	1.06	Present
Adult.....	500	885	Present	3.03	Present

A comparison of the two tables showing the results obtained in these groups of animals, representing different age limits, shows in the first place, that the polyuria which, as has been previously stated, becomes pronounced with the development of the glycosuria. The animals of the adult group show a higher degree of polyuria than do the younger animals; and they also show, with one exception, a uniformly higher percentage of glucose.

Although quantitative determinations of acetone were not made, we feel reasonably certain that the acetone output in the adult animals was distinctly in excess of that of the younger animals. Judging from the density of the precipitate of albumen the same statement may be made for this element of the urine.

The microscopic examination of the centrifugalized urines invariably showed that in the adult animals casts were far more numerous than in the young animals. The increase in the number of fatty casts was especially noticeable.

From the foregoing observations, the following deductions appear allowable:

1. Uranium nitrate when given subcutaneously in the dose of 6.7 mgs. per kilogram induces in the dog a series of changes which vary in their degree of severity.

2. The severity of these changes is determined by the age of the animal, the changes being more pronounced in adult animals and less pronounced in young animals.

3. The factors which determine these differences in the response of animals of different ages to the same quantity of uranium are at present undetermined.

THE EFFECT OF GRÉHANT'S ANESTHETIC<sup>2</sup> ON THE OUTPUT AND  
COMPOSITION OF THE URINE IN YOUNG  
AND IN ADULT ANIMALS

In the experiments that have been previously reported (1) in which this anesthetic was employed, the anesthetic was used in the full strength advised by its originator. In order to exclude the possibility of the anesthetic unduly depressing the circulation, in the present experiments the quantity of the anesthetic has been reduced to 60 per cent strength. In this strength the anesthetic was given to ten adult animals and eight young animals after they had been rendered nephritic by uranium.

The ten adult animals following the development of an anesthesia which was not profound became anuric and remained anuric throughout the experiments. The anuria was uninfluenced by the various diuretic substances which have been previously enumerated.

The rapidity with which the anuria develops has varied slightly in the different animals and is apparently dependent to some extent upon the depth of the anesthesia.

The following experiments illustrate this point:

*Experiment 4.* The animal was receiving 500 cc. of water daily. On the day of the experiment the animal had an output of urine of 1130 cc. Within forty minutes after the commencement of the anesthetic, which resulted in a complete anesthesia, the animal became anuric. During the course of the experiment no urine was obtained. At the commencement of the experiment the animal had a blood pressure of 95 mm. of mercury, and at its termination a blood pressure of 115 mm. of mercury.

Following the injection of 1 cc. of a 1 per cent solution of caffeine per kilogram, the general blood pressure was raised from 105 to 115 mm. of mercury and the oncometer pressure as indicated by a water manometer, showed a rise of 16 mm. of water.

*Experiment 7.* In the following experiment the animal was imperfectly anesthetized. During the operation he was constantly strug-

<sup>2</sup> Gréchant's anesthetic: The animal is given 0.25 cc. per kilogram of a 4 per cent solution of morphine. This is followed in half an hour by 10 cc. per kilogram of the following mixture: chloroform, 50 cc.; alcohol and water, each 500 cc.

gling. On the day of the experiment the output of urine was 1025 cc. For the first half hour period that the animal was under observation after the completion of the operation the output of urine was 2.7 cc. Following this period of diuresis the animal became completely anuric, and with the development of the anuria the struggling and other evidences of an imperfect anesthesia ceased.

At the commencement of the experiment the animal had a blood pressure of 105 mm. of mercury, and at its termination a blood pressure of 165 mm. of mercury. At no time during the experiment was there evidence of any over action of the anesthetic. The animal remained completely anuric to caffeine, theobromine, digitalin, 0.9 per cent sodium chloride and 2 per cent sodium chloride. The experiment shows that even though an adult animal may not be completely anuric at the commencement of an experiment, the changes in the kidney which are inaugurated by the anesthetic, progress with the anesthesia, and that with the development of a state of satisfactory surgical anesthesia an adult animal which has been anesthetized by Gréhan's anesthetic becomes anuric.

The young animals and puppies that had been rendered nephritic, glycosuric, and polyuric by the same quantity of uranium per kilogram that was employed for the adult animals, showed after the administration of Gréhan's anesthetic in 60 per cent strength a distinct difference in the effect of the anesthetic on the output and composition of the urine.

Although this group of young animals received the same quantity of Gréhan's anesthetic, and although they were allowed the same time in which to develop the aesthesia, they were not so completely anesthetized at the expiration of this period as were the adult animals. It was usually necessary to give these animals a small quantity of ether to complete the anesthesia. After a satisfactory state of anesthesia had been established, it was rarely necessary to administer the ether again. The animals remained satisfactorily anesthetized throughout the experiment, which usually lasted several hours.

The animals of this group show a distinct difference in the effect of the anesthetic on the urine flow. None of the members of the group were rendered anuric by the anesthetic, but, on the

other hand, they were distinctly diuretic before and after the introduction of various diuretic substances.

The urine of this group of diuretic animals showed after the anesthetic an increase in the quantity of glucose and in those animals in which acetone was not present in the urine prior to the anesthetic, after the anesthetic, acetone was invariably present.

The observation that has been made relative to the increase in the quantity of glucose in the urine after the anesthetic might be questioned on the ground that the increase in glucose was a relative rather than an absolute increase, e.g., that with the animals not so diuretic after the anesthetic as they were before, the fact that the urine became more concentrated would show a rise in the percentage of glucose.

To eliminate this possibility a series of normal animals were given Gréhant's anesthetic, and the changes induced in the composition of the urine were noted. (3) Following the anesthetic these animals developed a glycosuria, the percentage of glucose varying with the age of the animals. It seems therefore allowable to conclude that when Gréhant's anesthetic is given to a glycosuric animal the increase in the percentage of glucose in the urine is an absolute rather than a relative increase.

The following experiment will serve to show the difference in the output and composition of the urine in a young animal from that of the previously discussed adult animals.

*Experiment 18.* The animal was receiving 400 cc. of water daily. On the day of the experiment the output of urine was 780 cc. The urine contained 0.446 per cent glucose but no acetone. Following the anesthetic, the glucose increased to 0.99 per cent and an acetonuria developed. At the completion of the operation this animal had a blood pressure of 111 mm. of mercury and a urine flow of 1.4 cc. per ten minute interval.

During the course of the experiment, which lasted for three and a half hours, the animal was completely anesthetized and was freely diuretic to caffeine, digitalin and 0.9 per cent sodium chloride. At the termination of the experiment the animal had a blood pressure of 118 mm. of mercury and a urine flow of 4.5 cc. per ten minute interval.

## THE EFFECT OF MORPHINE-ETHER ON THE OUTPUT AND COMPOSITION OF THE URINE IN YOUNG AND ADULT ANIMALS

The young animals which received this type of anesthetic showed but slight differences in the output and composition of the urine and in their response to diuretics from the young animals receiving Gréhan's anesthetic. The following experiment shows the similarity in the response of these animals to morphine-ether.

*Experiment 17.* The animal was completely anesthetized. Prior to the anesthetic the animal was receiving 400 cc. of water. On the day of the experiment the animal had an output of urine of 790 cc. The urine contained 0.202 per cent of glucose and was free from acetone. Following the anesthetic the animal remained diuretic and had an output of urine of 2 cc. per ten minute interval before the use of any diuretic substance. During the course of the experiment the animal remained diuretic to caffeine and digitalin. The urine collected showed an increase in glucose to 0.501 per cent. The urine contained acetone.

The adult animals which were given morphine-ether differed from the adult animals receiving Gréhan's anesthetic in that they did not become anuric, but remained responsive to the same diuretic substances which when employed in animals anesthetized by Gréhan's anesthetic were found to have no diuretic value.

Two very old animals that were nephritic from uranium which were given morphine-ether, became anuric and the anuria was uninfluenced by caffeine, digitalin and 0.9 per cent sodium sulphate. These animals showed a pathological response on the part of the kidney which was similar to the pathology which develops in adult animals following Gréhan's anesthetic and which renders the animal anuric.

The following experiment shows the usual effect of morphine-ether as an anesthetic in adult animals.

*Experiment 16.* The animal was receiving 500 cc. of water daily. On the day of the experiment the output of the urine was 1015 cc. The urine contained 1.22 per cent of glucose. Acetone was present.



Following morphine-ether, which resulted in a complete anesthesia, the animal remained diuretic. During the first ten minute period, before the use of any diuretic substance, the output of urine was 3.4 cc. The animal was diuretic to both caffeine and digitalin. The urine obtained during the experiment showed an increase in glucose to 2.77 per cent.

CONCLUSIONS CONCERNING THE EFFECT OF GRÉHANT'S ANESTHETIC AND OF MORPHINE-ETHER UPON THE OUTPUT AND THE COMPOSITION OF THE URINE IN YOUNG AND IN ADULT DOGS NEPHRITIC FROM URANIUM

1. When given to adult animals, Gréhant's anesthetic produces a state of anuria which is uninfluenced by any of the diuretic substances that have been employed in this investigation.

2. When Gréhant's anesthetic is given to young animals, the animals remain diuretic and are responsive to the various diuretic substances. The urine collected during the anesthesia shows an increase in glucose over that induced by the injections of uranium before the anesthetic.

3. Adult animals when given morphine-ether have only occasionally developed an anuria. The animals in our series which have developed this condition have been old animals. In the animals anuric from morphine-ether the changes in the kidney are similar to those found in the kidneys of the animals anuric from Gréhant's anesthetic.

In general, the adult animals anesthetized with morphine-ether remain diuretic and are responsive to the different diuretic substances. The urine shows an increase in the percentage of glucose.

4. The young animals anesthetized with morphine-ether have without exception remained freely diuretic throughout the experiments. The urine collected during the experiments has shown a slight increase in the percentage of glucose. This increase, however, is less than the increase observed in the adult animals under the influence of the same anesthetic.

5. In conclusion, the age of the animal apparently exerts some influence over the toxicity of the anesthetic as did the same factor influence the toxicity of the uranium prior to the anesthetic.

THE EFFICIENCY OF VARIOUS DIURETICS IN URANIUM NEPHRITIS  
IN ANIMALS ANESTHETIZED WITH GRÉHANT'S ANESTHETIC  
AND WITH MORPHINE-ETHER

*The efficiency of various diuretics in animals anesthetized with  
Gréchant's anesthetic*

In considering the efficiency of these different diuretic substances in animals anesthetized with Gréchant's anesthetic, the nephritic animals, so far as their response to the diuretics is concerned, are found to arrange themselves into two groups: a diuretic group which is represented by the young animals; and an anuric group which consists of adult animals which had received the same quantity of the nephrotoxic substance per kilogram and the same amount of the anesthetic per kilogram as was received by the young animals.

In these animals the following diuretic substances were employed: caffeine, theobromine, digitalin, 0.9 per cent and 2 per cent sodium chloride, 0.9 per cent and 2 per cent sodium carbonate, 0.9 per cent sodium sulphate, and 0.9 per cent lithium chloride.

When these substances were employed in adult animals nephritic from uranium and anuric from Gréchant's anesthetic, they had no diuretic value. After having once become anuric these animals remained anuric.

The following experiments are representative of this group and demonstrate the inefficiency of substances as diuretics which in a normal animal or in a nephritic animal which has not become anuric, usually induce a distinct diuresis.

*Experiment 12.* On the day of the experiment this animal had an output of urine of 980 cc. Following Gréchant's anesthetic the animal became completely anuric, and remained anuric throughout the experiment. Caffeine induced a rise in general blood pressure of from 125 to 140 mm. of mercury without any change in kidney blood pressure. Digitalin induced a rise in general blood pressure of from 130 to 140 mm. of mercury with a rise in kidney blood pressure of 6 mm. of water. Sodium carbonate in 2 per cent solution, given in the quantity of 10 cc. per kilogram, caused a rise in general blood pressure of 13 mm. of mer-

cury and a rise in kidney blood pressure of 16 mm of water. The animal received 175 cc. of salt solution.

*Experiment 7.* This animal during the first half hour period of observation was diuretic. Following this initial stage of diuresis the animal became completely anuric and remained anuric, uninfluenced by the diuretics.

The animal's general blood pressure varied between 105 mm. of mercury at the commencement of the experiment to 165 mm. of mercury at its termination. At the commencement of the experiment the oncometer showed a kidney pressure of 25.3 cm. of water and at the termination a pressure of 32 cm. Caffeine induced a rise in blood pressure of 32 mm. of mercury and a rise in oncometer pressure of 16 mm. of water. Digitalin increased the general blood pressure 30 mm. of mercury, and the oncometer pressure 27 mm. of water, 0.9 per cent sodium chloride, 10 cc. per kilogram gave a rise in general blood pressure of only 5 mm. of mercury, while the oncometer showed an increase in pressure of 48 mm. of water, 2 per cent sodium chloride in the quantity of 10 cc. per kilogram caused a rise in general blood pressure of 17 mm. of mercury and a rise in kidney blood pressure of 27 mm. of water. The animal received 248 cc. of salt solution.

With the employment of other diuretic solutions in this group of anuric animals, such for instance as sodium sulphate and lithium chloride in 0.9 per cent solution, the same type of response was obtained in general blood pressure changes and in the local vascular changes in the kidney as were obtained from the salt solutions just described. Neither the type of salt nor the difference in the tonicity of the solutions which were employed induced any diuretic effect.

The following series of animals which did not become anuric from Gréhant's anesthetic were young animals. Prior to the anaesthetic they had been rendered nephritic by the same quantity of uranium per kilogram as was received by the adult animals; and later they received the same quantity of the anesthetic per kilogram as was received by the adult animals.

With this group of animals the experiments were conducted in a manner identical with the group just discussed; the same diuretic substances were employed; and they were given to the animals in this same quantity per kilogram.

The following experiment illustrates the difference in diuretic response of the two groups.

*Experiment 18.* The animal before the use of a diuretic had a flow of urine of 1.4 cc. per ten minute interval. Following caffeine, which induced a rise in general blood pressure of 6 mm. of mercury and in oncometer pressure of only 6 mm of water, the urine flow increased to 2.5 cc., digitalin produced a rise in general pressure of 6 mm. of mercury and in oncometer pressure of 15 mm. of water. The urine flow was increased from 1.1 cc. to 1.7 cc. per ten minute interval, following 0.9 per cent sodium chloride, which induced practically no change in general blood pressure but a rise in kidney blood pressure of 23 mm. of water; the urine flow increased from 2 to 4.5 cc.

A comparative study of these two groups of experiments in which the animals received Gréhan's anesthetic and in which one group becomes anuric and fails to respond to diuretics, while the other group does not become anuric and does respond to diuretics, fails to show any variations in the changes in general blood pressure which could account for the difference in the output of urine.

The changes in the oncometer pressure in the two groups show that the kidney vessels are responsive to substances acting peripherally on these vessels and that they also respond to changes in the general circulation. A general analysis, however, of the pressure changes in the kidney induced by the different diuretics in the two groups of animals shows that in the diuretic animals the changes in the kidney pressure are usually more pronounced than they are in the anuric animals. At the present time experiments are being conducted with the object in view of determining whether or not there is any constant difference in the vascular response of the kidney vessels in the two types of animals.

*The difference in the renal pathology of animals anuric and diuretic following Gréhan's anesthetic*

In conducting the pathological study of the kidneys from these two types of animals every precaution was employed to eliminate sources of error by using several fixing fluids for the tissue

from each experiment and by adhering to a uniform staining technique.

Tissue was fixed in 10 per cent formaline, Zenker's fluid, and in corrosive-acetic. The formaline and Zenker fixed tissue was imbedded in cellodin, while the corrosive-acetic fixed tissue was imbedded in paraffin. Cellodin sections were cut varying between 10 to 15 micra while the paraffin sections used for the comparative study were 6 to 8 micra in thickness. The stains employed were haematoxylin and eosin. In addition to this, frozen sections were made and stained for fat by Herxheimer's Scharlach R. method. As has been clearly shown by Bullard (4) fat droplets in considerable number may be demonstrated by this stain, when Herxheimer's modification is used, which would be missed by the simple alcoholic solutions of Scharlach R. and of Sudan. III.

The following pathological report should be considered as a summary of the essential differences in the kidneys of these two types of animals. A detailed discussion of the pathology will be published elsewhere.

In adult animals which had been rendered nephritic by uranium and killed by shooting, so as to eliminate the effect of the anesthetic, the kidney shows in the gross a severe congestion of the outer cortex and of the medulla. Between the cortico-medullary boundary zone and the superficial portion of the cortex there is a mid zone which appears distinctly pale as contrasted with other portions of the kidney.

In young animals which have been subjected to a similar experimental technique, this pale zone of the cortex is either absent, or is much less pronounced, while in general the cut surface of the kidney appears uniformly and severely congested.

The histological study of tissue from the kidneys of these two groups of animals which have not received an anesthetic shows a vascular reaction which is manifested by an engorgement of the glomerular vessels, but without any intra-glomerular or inter-tubular exudate. The epithelium of the tubules shows a shrinkage which gives to the lumen of the tubules an unusual prominence (figs. 1 and 2). Thus far the grosser pathological changes in

the two groups of animals are similar. The changes, however, differ very strikingly in this respect. The adult animals show a high degree of fatty degeneration in the tubules of the medullary rays and in the distal convoluted tubules, while tissue from the kidneys of the young animals show this fatty change to a much less extent, though when it develops the same tubules are involved. This is one of the striking differences in the pathological response of these two groups of animals.

When adult animals and young animals are anesthetized by the same quantity of Gréchant's anesthetic per kilogram, the gross and microscopic pathology of the kidneys shows the following differences.

In the adult animals the cut surface of the kidney does not show such a severe congestion and the mid-cortical zone of paleness has perceptibly increased in distinctness and extent. The microscopic study shows that the fatty changes which have been induced by the uranium have been very greatly increased by the anesthetic and that the pale zone of the cortex is, in part, due to these changes. As a result of the effect of the anesthetic the epithelium of the tubules of the labyrinth, especially the proximal convoluted tubules, has become acutely swollen, with a part or complete occlusion of the tubular lumen. The epithelium is in various stages of degeneration and necrosis (fig. 3). The rapidity with which this swelling develops is remarkable.

In the kidneys of the young animals following Gréchant's anesthetic, the same type of changes are induced, but to a much less extent. Thus there develops in the cortex a pale zone which is much less extensive than in adult animals and microscopically the fatty changes in this zone are found to be less pronounced. Equally noticeable as the difference in the fat content (microscopically demonstrable) is the difference in the degree of involvement of the tubules of the labyrinth. In the young animals which have remained diuretic the severe grade of swelling of the epithelium which was so noticeable in the adult animals is comparatively slight or absent (fig. 4).

*The difference in the renal pathology of animals anuric and diuretic following morphine-ether*

With two exceptions these animals following morphine-ether remained diuretic.

The diuretic group was composed of both young and adult animals. In this group uranium induced the same type of changes as have been described in the animals which were given Gréhant's anesthetic. When, however, morphine-ether was employed as the anesthetic the fatty changes which had been induced by the uranium were not increased to a degree comparable to the increase in these changes which followed Gréhant's anesthetic, and also the acute swelling of the epithelium of the tubules of the labyrinth was either absent or much less marked (fig. 5).

The exceptions to this statement are found in two animals which were very old; and these animals following morphine-ether showed a pathological response on the part of the kidney which was comparable to the pathology seen in the kidneys of the adult animals which received Gréhant's anesthetic, and like these animals they were rendered anuric.

GENERAL DISCUSSION OF THE EXPERIMENTAL DATA

The metabolic disturbance which is induced by uranium and which in part is characterized by the development of a glycosuria is usually explained by assuming that this substance like hydrocyanic acid induces the glycosuria by lessening internal respiration.

In the experiments conducted by Chittenden and Hutchinson (5) on the influence of uranium salts upon the activity of certain ferments they were able to show that the nitrate exerted an inhibitory effect upon the ferment action of saliva and of pepsin. This inhibition was induced by the nitrate in very high dilutions. An inhibitory effect on the ferment action of saliva was brought about with dilutions of the salt in 0.0001 to 0.003 per cent strength while the inhibition of the proteolytic action of pepsin required the use of the salt in stronger solutions. The action of this fer-

ment was inhibited when uranium nitrate was used in the strength of 0.01 per cent, and all action ceased when the strength of the solution increased to 0.5 per cent.

It is possible that uranium nitrate exerts a similar inhibitory effect upon the action of the oxidative enzymes of the cell, and that through this action internal respiration, even in the presence of abundant oxygen, is interfered with. The lessened oxidation so induced would explain the glycosuria that is constantly seen following uranium injections.

Granting that such a hypothetical explanation for the uranium glycosuria be true the experiments which have been conducted in this investigation would tend to show that the oxidative capacity of the young animals is greater than that of the adult animals, for when these two groups of animals have received uranium nitrate in the same quantity per kilogram the percentage of glucose in the urine of the young animals is much less than is the percentage in the urine of the adult animals.

When we consider the unusual demand for activated oxygen which likely exists in the tissues of rapidly growing young animals, we see that such an assumption concerning the relative oxidative capacity of young and adult animals is not especially visionary.

In addition to the evidence of a disturbed metabolism that is manifested by the appearance of glucose in the urine, the use of uranium also induces fatty changes in the kidney and in the liver. So far as their severity is concerned, these changes bear the same relation to the age of the animal as was seen to exist for the percentage of glucose in the urine. The fatty changes in the liver and in the kidney are much more pronounced in the adult animals than they are in the young animals.

When these nephritic and glycosuric animals are given an anesthetic, those changes, of whatever origin they may be, which have been induced by the uranium injections become greatly augmented. The percentage of glucose in the urine is increased, the fatty changes in the kidney and in the liver are more marked, and the nephritis is so increased in severity that certain of the animals rapidly develop an anuria.



These changes which are induced by the anesthetic are also influenced by the age of the animal, and they are found to be more pronounced in the adult animals than they are in the young animals. An observation similar to this has been made by Whipple (6), who was able to show that chloroform induced but slight fatty degeneration and liver necrosis in young pups, while in adults a marked central hyaline necrosis of the liver was induced.

Not only does the age of the animals influence the severity of the action of the anesthetic but the type of anesthetic employed also aids in determining the severity of its effect.

Of the two anesthetics which were used in the experiments Gréhan's anesthetic gave more evidence of being toxic and had more effect in increasing the severity of the nephritis and in establishing a condition of anuria.

The active anesthetic ingredients in Gréhan's anesthetic are chloroform and alcohol, and from the observation which has just been made, it would appear that of the two anesthetics, Gréhan's and morphine-ether, Gréhan's which contains chloroform and alcohol is far more toxic in a nephritis than ether. This would be especially true in those nephritides in which the parenchymatous element of the kidney is principally involved.

A study of the response of the nephritic kidney to diuretics, shows that the efficiency of a given diuretic very largely depends upon the age of the animal, and upon the anesthetic which has been employed. Thus young and adult animals nephritic from uranium when anesthetized with morphine-ether remain diuretic to the substances which have been used in the experiments. The same statement holds true for the young animals which were anesthetized with Gréhan's anesthetic. When, however, an adult animal nephritic from uranium is anesthetized with Gréhan's anesthetic, the animal becomes anuric and remains refractory to the diuretic substances which have induced a free diuresis in the other animals. A similar condition of anuria with a failure to respond to the diuretics has been observed in two old animals anesthetized with morphine-ether.

The renal pathology which is characteristic of this anuric group consists in a rapid swelling and necrosis of the epithelium, especially of the proximal convoluted tubules.

A physiological study of this anuric group shows that the anuria is not dependent upon a general condition of low blood pressure. The degree of response of the renal vessels to substances acting locally in the kidney and through changes in the general blood pressure is certainly sufficient to influence diuresis in a normal kidney. When compared with the degree of response on the part of the renal vessels in the diuretic animals it would appear that it was sufficient to induce diuresis in these anuric animals. In the anuric group, however, with the pronounced swelling of the epithelium which is constantly present, the quantity of blood reaching the glomeruli and the rate of blood flow through the kidney must be to some extent interfered with. This is likely a part of the explanation for the anuria.

With this cause for the anuria in mind, in these animals which had remained anuric to diuretics such as caffeine and 0.9 per cent sodium chloride, hypertonic salt solutions were employed with the object in view of inducing a shrinkage of the swollen cells and by removing any obstruction to the renal circulation and at the same time by removing any mechanical obstruction to the flow of the urine through the partly or completely occluded tubules to induce a diuresis.

When such hypertonic solutions were used it was found possible to induce a shrinkage of the epithelium (fig. 6). With such a change in the size of the epithelial cells which would tend to decrease the volume of the kidney, the oncometer showed an increase in kidney volume. This rise in kidney pressure is likely due to the dilator effect of the hypertonic solutions on the renal vessels. With this change in the vessels, increasing the quantity of blood reaching the kidney and with the epithelium shrunken the circulation through the kidney should be distinctly improved. Even through such a change in the renal circulation had apparently been induced, the animals remained anuric. Whether or not we have in this anuric type of nephritis, as suggested by Pearce (7), a condition of the vessels which allows dilatation, but

in which the vessels are so influenced by the anesthetic as to cause decreased glomerular permeability, it is difficult to say.

In three animals of this anuric group the use of salt solution caused a well marked inter-tubular exudate to be produced. This observation would tend to decrease the probability of Pearce's explanation for the anuria, for it shows that some of the vessels are permeable to salt solutions.

The salt solutions were also employed as diuretics for the purpose of rendering the blood more hydraemic and at the same time of decreasing its viscosity. These changes in the blood had no effect in re-establishing a urine flow.

In conclusion, it would appear that in a uranium nephritis when following an anesthetic the epithelium becomes severely damaged, the animal develops an anuria uninfluenced by diuretics which increase the efficiency of the vascular mechanism of the kidney and which so alter the composition of the blood as to favor diuresis.

The investigation would, therefore, tend to favor the conception of the kidney's activity being more dependent upon the secretory capacity of its cells than upon any mechanical conception of the action of the vascular mechanism of the kidney.

#### CONCLUSIONS

1. In dogs in which an acute nephritis has resulted from the subcutaneous administration of uranium nitrate in the dose of 6.7 mgs. per kilogram the severity of the nephritis, of the glycosuria, and of the polyuria is influenced by the age of the animal. The changes in the kidney and in the urine are more marked in adult animals than they are in young animals and in puppies.

2. When such nephritic, glycosuric, and polyuric animals are anesthetized by Gréhan's anesthetic or by morphine-ether, the severity of the nephritis is increased and the output and composition of the urine is changed.

3. The increase in the severity of the nephritis is more marked from the use of Gréhan's anesthetic, the active anesthetic ingredients of which are chloroform and alcohol, than from morphine-ether.

4. In addition to the fact that the type of anesthetic influences the renal pathology the age of the animal also aids in determining the severity of the changes. The changes are more pronounced in adult and old animals than in young animals and puppies.

5. Following the anesthetic these nephritic animals either become anuric or remain diuretic.

6. In the anuric group which is principally represented by the adult animals which have received Gréhant's anesthetic the condition of anuria is uninfluenced by various diuretics.

7. The failure of this group to respond to diuretics is more likely due to the destruction of the epithelium of the kidney than to any physiological or anatomical change in the vascular element of the kidney.

8. Those animals that following the anesthetic are not rendered anuric are responsive to the same diuretic substances which in the anuric group have been shown to have no diuretic value.

9. In this diuretic group of animals regardless of whether the anesthesia has been induced by Gréhant's anesthetic or by morphine-ether, the epithelial involvement of the kidney is much less severe than in the anuric group.

In conclusion I desire to acknowledge my indebtedness to Dr. J. P. Jones for his valuable aid in conducting the experiments.

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## EXPLANATION OF PLATE

1. Kidney of an adult animal, nephritic, glycosuric and polyuric from uranium. The epithelium is shrunken and gives to the lumen of the tubules undue prominence. The epithelium shows an early vacuolation. The glomerular vessels fill the capsular space. The tubules contain granular detritus.

2. Kidney of a puppy, nephritic, glycosuric and polyuric from uranium. The changes in general are similar to those seen in figure 1.

3. Kidney of an adult animal following Gréchant's anesthetic given in 60 per cent strength. Prior to the anesthetic the animal was excessively polyuric. Following the anesthetic, within forty minutes, the animal became completely anuric. The figure shows the acute swelling of the epithelium with an occlusion of the lumen of the tubules. The epithelium of the beginning of the collecting tubules is spared. These tubules remain open. The glomerular vessels do not fill the capsular space. This figure should be compared with figs 1 and 4.

4. Kidney of a puppy following Gréchant's anesthetic. The animal had been rendered nephritic with the same quantity of uranium per kilogram as had been used for the adult animal, figure 3. The puppy was anesthetized with the same quantity of Gréchant's anesthetic per kilogram as was the adult animal. The figure shows the absence of epithelial involvement. The tubules are not occluded. The glomerular vessels fill the capsular space. The animal was freely diuretic.

5. Kidney of an adult animal following morphine-ether as an anesthetic, nephritic from uranium. The animal remained freely diuretic. The figure shows the absence of the acute swelling of the epithelium which was the most characteristic change in the figure from the adult animal anesthetized with Gréchant's anesthetic.

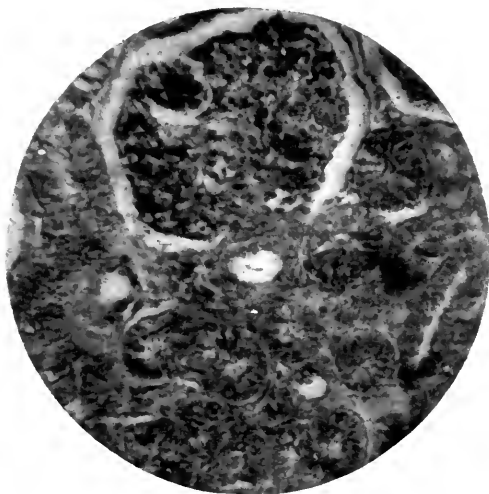
6. Partial shrinkage of the epithelium in an adult anuric animal from the use of hypertonic salt solution. The epithelium is in an advanced stage of degeneration. The glomerular vessels fill and distend the capsule and are histologically well preserved. The animal remained anuric.



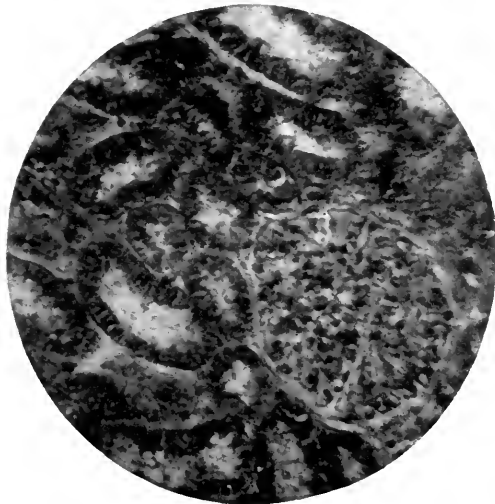
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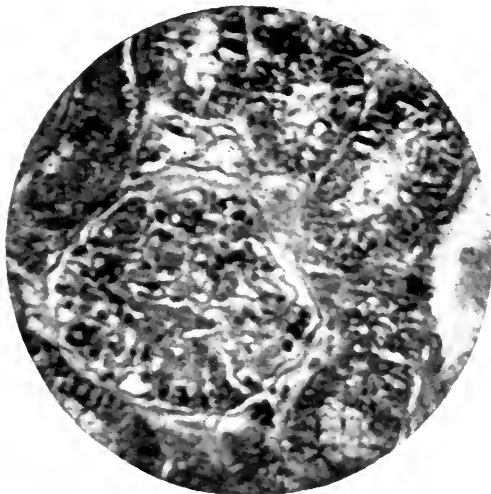
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## THE EFFECT OF VARYING TONICITY ON THE ANAPHYLACTIC AND OTHER REACTIONS OF PLAIN MUSCLE

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It was shown by Friedberger and Hartoch (1), that a preliminary injection of saturated sodium chloride solution enabled sensitised guinea-pigs to survive a reinjection of the sensitising antigen, which was sufficiently large to be uniformly fatal to identically sensitised control animals. Friedberger and Langer (2) have shown that sodium chloride given by the mouth in sufficient quantity has a similar protective effect. Friedberger and Hartoch attributed the effect of the salt to the well-known inhibiting action of hypertonic solutions on the fixation of complement, as studied *in vitro*. They found, indeed, that the mitigation of the symptoms following reinjection was attended by a diminution in the loss of complement, which accompanies a normal anaphylactic reaction. Since, according to Friedberger's theory, the complement plays an essential part in the production of "anaphylatoxin," any influence which inhibited complement-fixation would, on this theory, prevent the appearance of anaphylactic symptoms. Doubt has been cast on this explanation from more than one quarter. Bornstein (3) pointed out that intravenous injection of salt in such quantities caused a considerable dilution of the blood and increase of its volume, by the entry of water from the tissues. He regarded this as an important factor in the protective action. Ritz (4) showed that a preliminary salt injection protected against the action of "anaphylatoxin" prepared *in vitro*, which, on Friedberger and Hartoch's view, should not be affected. The demonstration of the anaphylactic reaction of isolated plain muscle, by Schultz (5), and by

myself (6), occurring under conditions in which the action of complement and the production of poisonous cleavage products appeared to be excluded, suggested to me that the protective action of salt injections might be due to a depression of the responsiveness of the plain muscle. By the use of isolated organs this possibility could be tested under conditions of ideal simplicity.

#### METHOD

As in my former experiments the uteri of young virgin guinea-pigs have been used throughout. Survivors from the standardisation of antitoxic (horse) serum have been used in all the experiments on anaphylactic response. Both horns of the uterus have been used in all experiments of this nature, the reaction of one being tested in Ringer's solution containing the usual 0.9 per cent of sodium chloride, to control the degree of sensitiveness, and the other being suspended in a similar solution, to which a further amount of sodium chloride has been added, in proportions varying in the different experiments. In some cases a preliminary perfusion of the uterus was carried out, as described in my previous paper. As perfusion fluid either the normal or the hypertonic Ringer's solution was used. The perfused horns were then transferred to separate vessels, one containing the normal, the other the hypertonic solution, kept warm and oxygenated. They were then tested in succession, each in the same volume of its appropriate solution. The choice of hypertonic or normal Ringer's solution for perfusion had no influence on the result, which was solely determined by the composition of the fluid in which the test was made. Identical results were obtained without preliminary perfusion. Care was also taken to perform the tests in the opposite orders in different experiments, so as to exclude any possible difference due to keeping one horn longer than the other in the solution. The temperature of the test solutions was kept uniform at 38°C. with special care. The use of the two horns of the uterus affords an opportunity of obtaining two anatomically and physiologically identical strips of uninjured plain muscle, such as can be obtained from no other organ.

The exact similarity of the curves obtained when the two horns were tested successively in solutions of the same composition, showed that any marked difference in the responses obtained with the two horns might with confidence be attributed to the only deliberate variation of the conditions, viz., the composition of the fluid in which the organ was suspended.

The Ringer's solution used for the controls was made up according to one of two formulae. The first was that which I used in my previously published experiments on the anaphylactic reaction, being Locke's formula, with the exception that the calcium content was reduced. It is as follows: NaCl, 0.9; KCl, 0.042; CaCl<sub>2</sub>, 0.012; NaHCO<sub>3</sub>, 0.05; dextrose, 0.1; water (distilled in glass), 100. This will be referred to as Ringer 1.

The second was modified from a formula given by Tyrode (7), and has proved an extremely effective medium for preserving the excitability of the isolated guinea-pig's uterus, without the spontaneous development of high tonus or large rhythm. It may be mentioned incidentally that it serves admirably for the method of standardising pituitary extracts which Laidlaw and I recently described (8). It is essentially Locke's solution, with the addition of a small proportion of magnesium chloride. The magnesium appears to possess a marked power of restraining the development of irregular automatic rhythm. The formula is: NaCl, 0.9; KCl, 0.042; CaCl<sub>2</sub>, 0.024; MgCl<sub>2</sub>, 0.0075; NaHCO<sub>3</sub>, 0.05; dextrose, 0.1; water (glass distilled), 100. This is referred to as Ringer II.

Neukirch (9) has used Tyrode's solution with like effect for preserving the regular rhythm and excitability of isolated intestinal loops from the rabbit.

The results obtained with these two Ringer's solutions, with varying proportions of additional sodium chloride, have been of the same general type.

#### MODIFICATION OF THE ANAPHYLACTIC RESPONSE

Friedberger and Hartoch's protective action was obtained by injecting as much as 1 cc. of saturated sodium chloride intra-

venously into a small guinea-pig (200 to 250 grams). Taking the concentration as 37 per cent, and assuming 15 to 20 cc. as the blood-volume of such a guinea-pig, it is easy to calculate that the injection must raise the sodium chloride in the blood to as much as 3 per cent. Such a concentration would, of course,

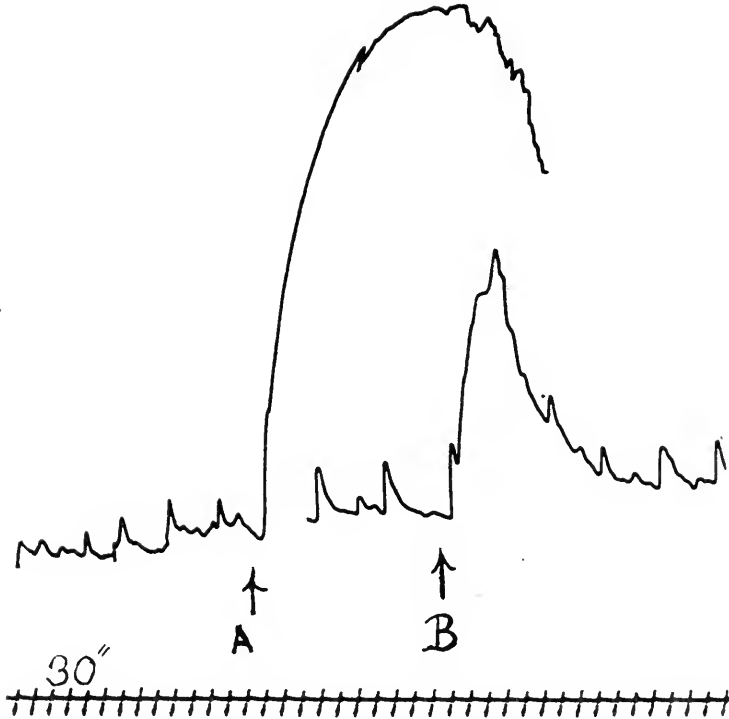


FIG. 1. UTERUS OF VIRGIN GUINEA-PIG (BOTH HORNS)

Sensitisation 1/500 cc. diphtheria antitoxic (horse) + 1 test dose toxin: eighteen days. Perfused with Ringer I. Bath volume (in all experiments illustrated) 250 cc. First horn in Ringer I, second horn in Ringer I + 0.2 per cent NaCl (1.1 per cent in all). At A and B 0.5 cc. horse-serum added to bath.

be only momentarily attained; but it is probable that the injection of antigen in such experiments is made while the tonicity of the blood is still at such a level that the isolated plain muscle could not long survive in an equivalent saline solution. It was quite unnecessary, however, to try the effect of such concen-

trated solutions on the response of the isolated organ, since a few experiments made it clear that a comparatively slight increase in the saline content of the fluid very markedly depressed the extent of the anaphylactic reaction.

Figure 1 shows the effect of raising the sodium chloride content from 0.9 to 1.1 per cent, 2 grams of salt being added to 1 liter of the Ringer's solution. Figure 2 shows a similar pair of

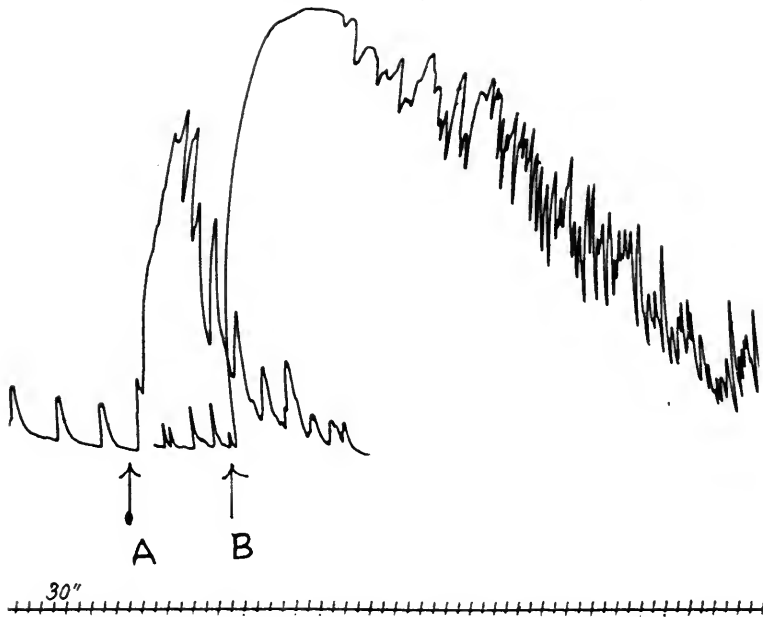


FIG. 2. UTERUS OF VIRGIN GUINEA-PIG (BOTH HORNS)

Sensitisation 1/45 cc. antitoxin + 1 test dose toxin: sixteen days. Perfused with Ringer I + 0.3 per cent NaCl (1.2 per cent in all). First horn in 1.2 per cent NaCl Ringer, second in normal Ringer I. At A and B 0.5 cc. horse-serum.

responses, taken in the reverse order, from one horn in Ringer's solution with 1.2 per cent NaCl, the other in the ordinary solution with 0.9 per cent NaCl. A rise to 1.3 per cent of sodium chloride produced almost complete extinction of the response to the antigen in the case illustrated in figures 3 and 4. Figure 4 illustrates another point of some importance. After the dose of antigen had been given at A, with minimal effect, the solution

was run off, and after repeated washing of the uterus with fresh quantities of the hypertonic solution, the bath was filled with the ordinary Ringer's solution, containing 0.9 per cent NaCl. This change produced a small rise of tonus, with the cause of which we shall deal in a later section. Here it is only necessary to mention that it has no connexion with the previous addition of antigen. A normal uterus, in the absence of any drug, shows a similar temporary increase of tonus when transferred from a

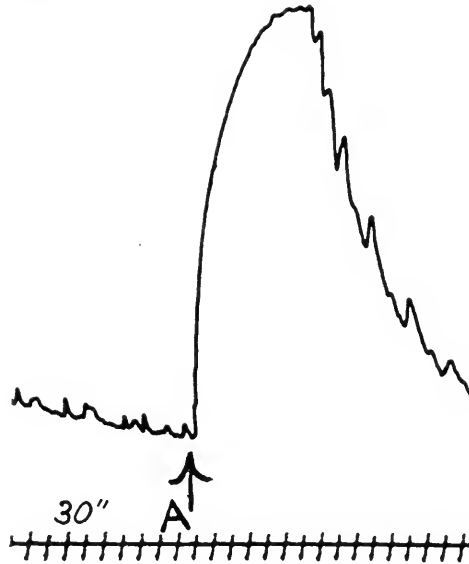


FIG. 3. UTERUS OF VIRGIN GUINEA-PIG

Sensitisation 1/600 antitoxin + 1 test dose: seventeen days. Perfused with Ringer I. At A 0.5 cc. horse-serum.

solution of higher to one of lower tonicity. After this change a further dose of the antigen was given at B, and was completely ineffective, although the control horn, receiving its first dose in the same solution, gave a typical anaphylactic response (fig. 3). This observation has been made repeatedly and always with the same result; though the tonicity of the solution was raised to such a point that addition of a full dose of antigen caused practically no contraction, the plain muscle was none the less effec-

tively desensitised to further doses, given in a solution in which contraction, apart from such previous treatment, occurs. Mere preliminary suspension of the organ in, or even prolonged perfusion with hypertonic Ringer, has no depressant effect on its subsequent response to the antigen in normal Ringer. The desensitisation can, therefore, only be attributed to the previous dose of antigen, and we are driven to the conclusion that the union of fixed antibody with antigen, and consequent desensitisation, is unhindered by the comparatively small increase of tonicity, which suffices practically to obliterate the contraction which normally results from such union. This is in line with the obser-

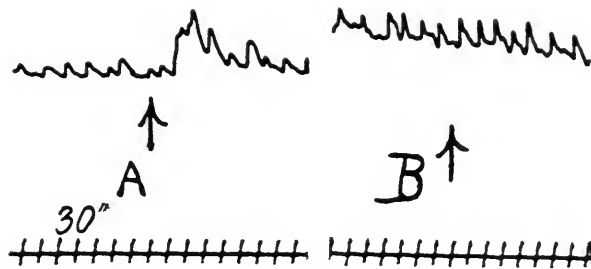


FIG. 4. SAME EXPERIMENT AS FIGURE 3.

Second horn in Ringer I + 0.4 per cent NaCl (1.3 per cent in all). At A 0.5 cc. horse-serum. Wash out several times with hypertonic and then change to normal Ringer I, to which, at B, add 0.5 cc. horse-serum.

vation of Ritz, that injection of strong saline protects against "anaphylatoxin" prepared *in vitro*, and leads to the expectation that the effect of added salt is to depress the responsiveness of the muscle to motor stimuli in general. In the following section it will be shown that this is, indeed, the case. Two further points, however, first need mention.

Firstly, the depressant effect is not due to excess of sodium ions in relation to others. If the concentration be increased by making up the Ringer's solution with a smaller proportion of water, while retaining the normal relation of the saline constituents to one another, the effect is identical with that produced by adding an extra quantity of sodium chloride.

Secondly, the customary solution with 0.9 per cent NaCl,

though it presumably represents approximately the saline composition of the body fluids, does not furnish the condition for maximum responsiveness of the isolated plain muscle. If we



FIG. 5. UTERUS OF VIRGIN GUINEA-PIG (BOTH HORNS)

Sensitisation 1/400 antitoxin + 1 test dose: fourteen days, Not perfused. First horn in Ringer II, second horn in Ringer II diluted with one-eighth volume of water. At A and B 0.01 cc. horse-serum.

regard the responsiveness in this solution as representing the normal condition, a supernormal excitability is obtainable by lowering the concentration of the bath by addition of distilled water. The increase of anaphylactic response produced by thus



lowering the concentration of sodium chloride from 0.9 to 0.8 per cent, and that of the other constituents in proportion, is illustrated in figure 5. In experiments on the whole guinea-pig some observers have found that a minute reinjection of antigen, sufficient to produce only a small rise of temperature when given in a small volume of saline, will cause death if given in a large volume of distilled water—Thiele and Embleton (10). The observation involves the response of other cells, as well as those of plain muscle, but the parallelism with the effect on isolated plain muscle is suggestive.

#### MODIFICATION OF THE RESPONSE OF NORMAL PLAIN MUSCLE

It was shown above that the modification of the anaphylactic reaction by altered concentration of the Ringer's solution is apparently due to the altered responsiveness of the muscle fibers, and not to inhibition or facilitation of the antigen-antibody reaction which furnishes the stimulus. This is confirmed when the effect of similar changes on the action of stimulant drugs, such as  $\beta$ -iminazolyethylamine or pituitary extract, is studied. With these we can study the phenomenon to much better advantage, since, as Laidlaw and I have shown, the plain muscle of the guinea-pig's uterus gives a very uniform series of responses to a succession of equal doses of these substances, provided that each dose is thoroughly washed away, and the normal condition of minimum tonus regained, before the next dose is added. I have used  $\beta$ -iminazolyethylamine for these experiments, as eliciting the more rapid and, on the whole, more uniform response: but pituitary extract, or pilocarpine, or, indeed, any stimulant drug, to which the muscle does not rapidly acquire tolerance with successive doses, will show the effect. It is necessary to emphasise this, since the resemblance between the action of iminazolyethylamine and certain features of the anaphylactic response might give the impression that the phenomenon with which we are dealing was peculiar to effects of this type.

Figure 6 shows the modification of the effect of a just maximal dose of iminazolyethylamine, produced by adding increasing

doses of sodium chloride to the 250 cc. of Ringer's solution (II) with which the bath was uniformly filled. It will be seen that the change from 1.3 to 1 per cent NaCl restores the activity almost to the original level, and that the restoration is practically complete on subsequently changing to solution of the original concentration, with 0.9 per cent sodium chloride.

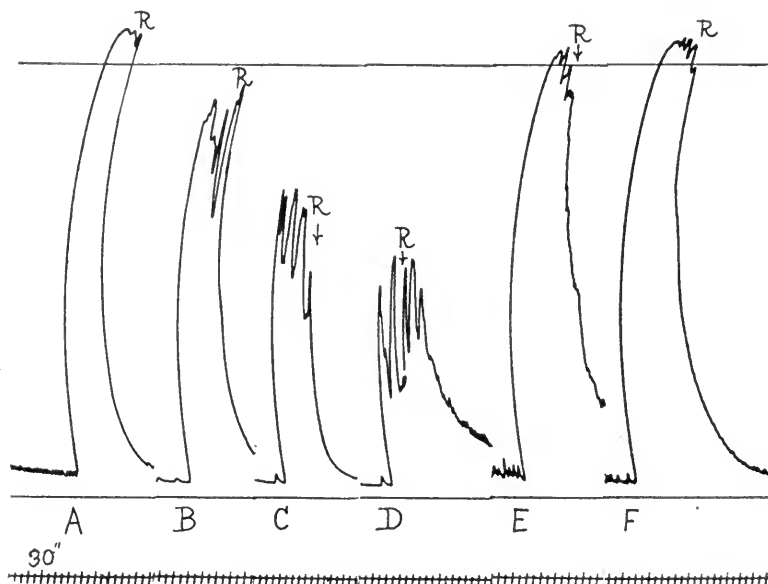


FIG. 6. HORN OF UTERUS OF NORMAL VIRGIN GUINEA-PIG (ALSO IN FIGS. 7-11)

Dose in each case 0.05 mgm.  $\beta$ -iminazolyethylamine. At A uterus is in Ringer II with 0.9 per cent NaCl (normal), B 1.1 per cent, C 1.2 per cent, D 1.3 per cent, E 1 per cent, F 0.9 per cent (normal). At R, R (in this and subsequent figures), change of Ringer's solution.

In the previous section it was noted that the depressant action is not due to increasing the proportion of sodium ions. It has now to be added that increase of the osmotic pressure of the solution by adding another electrolyte, such as sodium sulphate, or a non-electrolyte, such as cane-sugar, is equally effective in depressing the response of the plain muscle. No difference could be detected between the effects of sodium chloride and sodium

sulphate added in equimolecular proportions. The effect of adding 2 per cent of cane-sugar is illustrated in figure 7.

It was found, as expected, that the sensitiveness of the plain

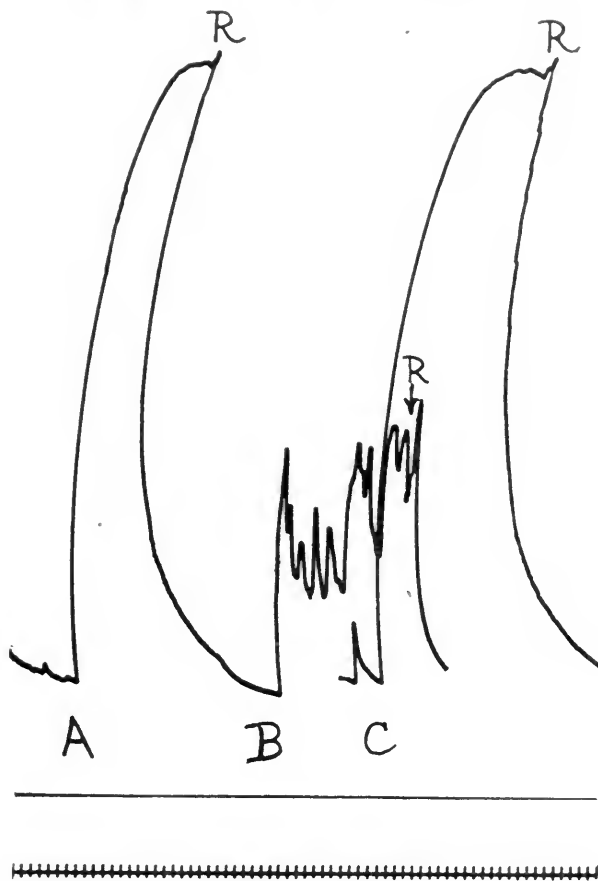


FIG. 7. DOSE IN EACH CASE 0.05 MGM.  $\beta$ -IMINAZOLYLETHYLAMINE

At A and C uterus is in normal Ringer II, at B in the same with the addition of 2 per cent cane sugar.

muscle to iminazolyethylamine was increased when the concentration of the Ringer's solution was lowered by addition of distilled water. A very small change of concentration produced a well-marked change of excitability. Figure 8 shows the effect

of four successive, equal, submaximal doses of iminazolyethylamine. The first two contractions were produced with the uterus in the ordinary undiluted Ringer's solution (II). The

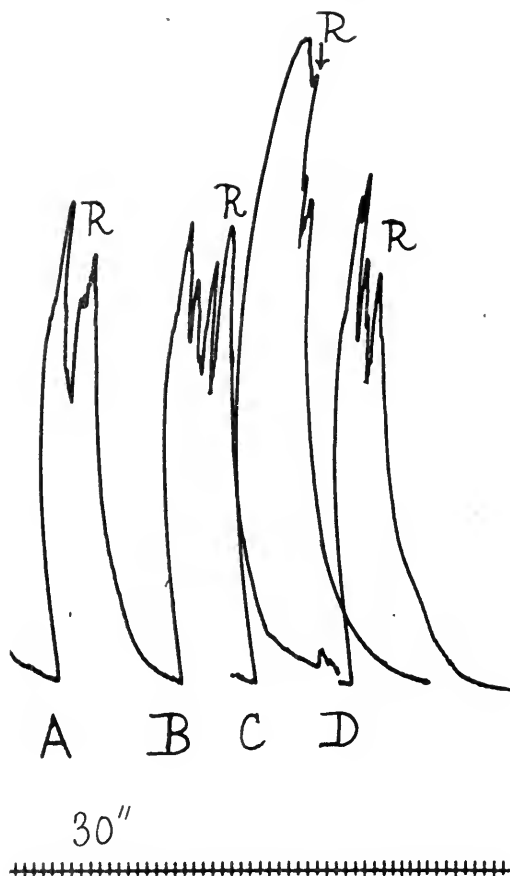


FIG. 8. DOSE IN EACH CASE 0.01 MGM.  $\beta$ -IMINAZOLYLETHYLAMINE

At A, B, and D, uterus is in normal Ringer II (with 0.9 per cent NaCl), at C in the same diluted with one-seventeenth volume of distilled water (reducing NaCl to 0.85 per cent).

dose producing the effect at B was washed away as usual, and the standard volume of 250 cc. fresh solution run in. To this 15 cc. of distilled water were added, bringing the concentration

of NaCl to about 0.85 per cent and lowering that of the other constituents in proportion. The volume in the bath was then again brought to 250 cc., and, when the muscle had returned

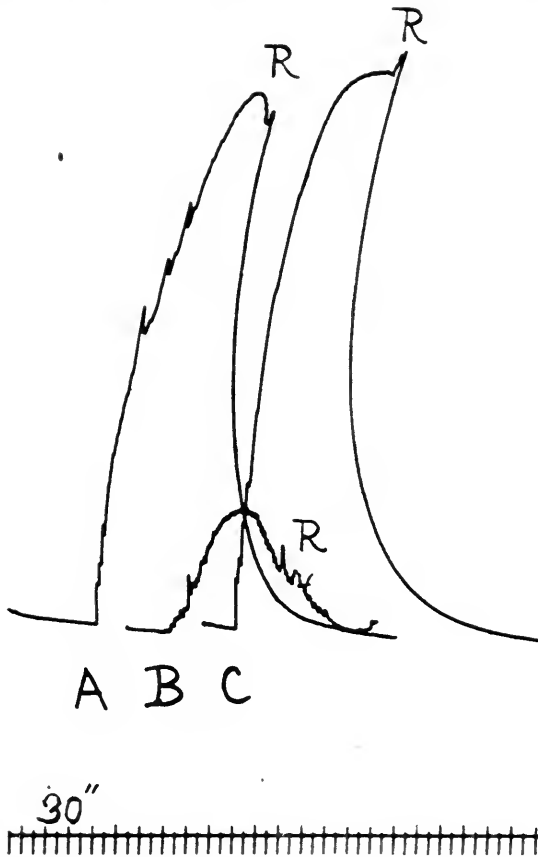


FIG. 9. DOSE IN EACH CASE 0.002 MG.  $\beta$ -IMINAZOLYLETHYLAMINE

At A and C uterus is in Ringer II, reduced by dilution to 0.75 per cent NaCl; at B in the same diluted to 0.8 per cent NaCl.

to the condition of minimum tonus, the third dose was given at C, producing a much larger effect. A change back to the ordinary concentration then restored the excitability to the original level, as shown at D.

Figure 9 shows the effect of a similar change, made at a lower level of concentration; it will be observed that in this case an extremely small dose (0.002 mgm.) has to be used in order to avoid producing a supramaximal effect in the solution containing 0.75 per cent NaCl.

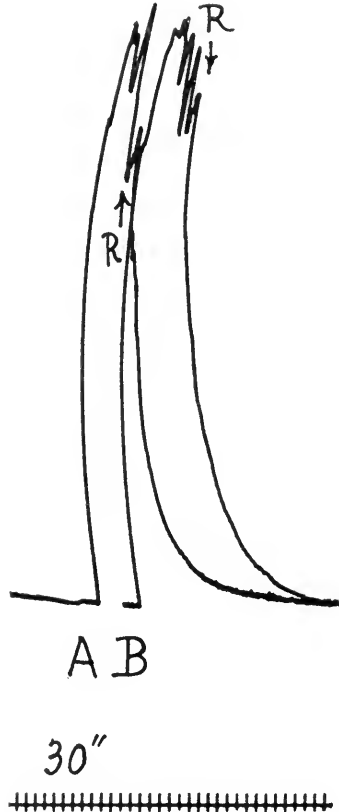


Fig. 10. DOSE IN EACH CASE 0.01 MGM.  $\beta$ -IMINAZOLYLETHYLAMINE

At A uterus is in normal Ringer II, at B in the same diluted with one-eighth volume of 12 per cent cane sugar.

The effect of dilution is due to the lowering of the tonicity of the solution. A diminution of the concentration in the electrolytes, within the limited range with which we are dealing, has no effect, provided that the osmotic pressure of the solution is

maintained by adding an equivalent amount of a non-electrolyte, such as cane-sugar. Figure 10 shows the equal effects of two doses of iminazolyethylamine (0.01 mgm.), given when the bath was filled respectively with ordinary Ringer's solution, and with

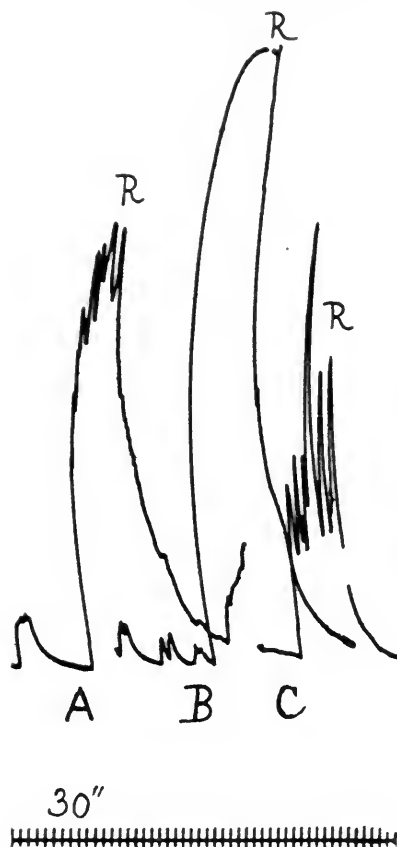


FIG. 11. DOSE IN EACH CASE 0.005 MGm.  $\beta$ -IMINAZOLYLETHYLAMINE

At A and C uterus is in normal Ringer II, at B in the same diluted with one-eighth volume of 2 per cent urea.

Ringer's solution of which the saline content had been reduced from 0.9 to 0.8 per cent NaCl by adding one-eighth of its volume of a 12 per cent (isotonic) solution of cane-sugar in distilled water. On the other hand, a 2 per cent solution of urea, which has about

the same osmotic pressure as Ringer's solution, acts as a diluent like distilled water (fig. 11). This, again, is in accordance with the supposition that the effect is due to change in the tonicity of the solution, since urea, to which the cell-membranes are perfectly permeable, is osmotically indifferent where living cells are concerned.

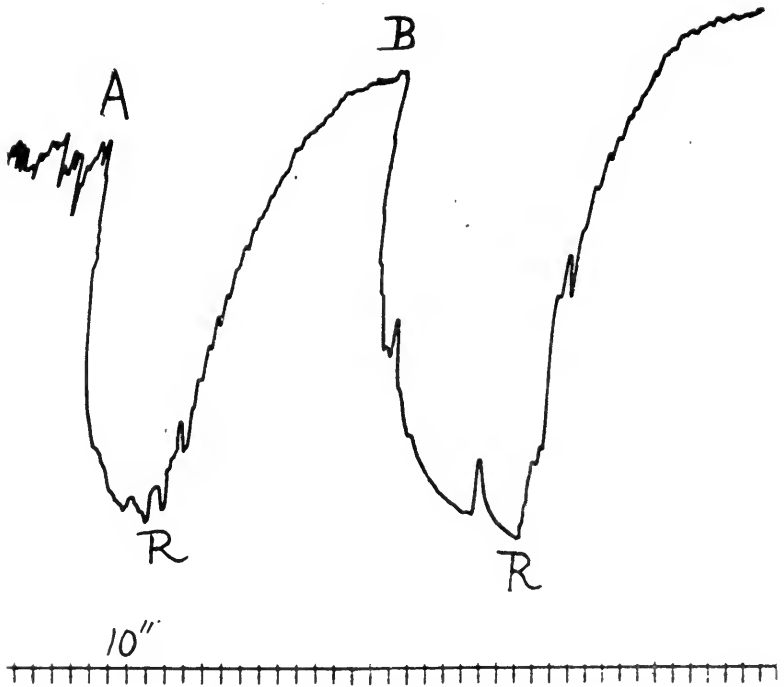


FIG. 12. HORN OF UTERUS OF VIRGIN CAT IN 250 CC. RINGER II

At A, 0.002 mgm. adrenaline. R, change to fresh Ringer. At B — 1 cc. 25 per cent NaCl (raising NaCl concentration from 0.9 to 1 per cent).

A fall in the tonicity of the bathing-fluid, if sufficiently large and sudden, is itself an efficient stimulus. Thus the addition of 30 cc. of distilled water to a bath containing 250 cc. of Ringer's solution usually provokes a contraction of the plain muscle suspended therein. A more marked contraction is produced by adding 50 cc. of water, which reduces the saline concentration



from 0.9 to 0.75 per cent NaCl. Indeed, the effect of a stimulant drug, such as iminazolyethylamine, may be closely simulated by thus suddenly reducing the concentration of the fluid; when the requisite amount of salt is added to restore the original concentration, relaxation occurs, as when the stimulant drug is washed away by fresh solution. It may be stated, therefore, in general terms, that a fall in the tonicity of the bath excites tonic

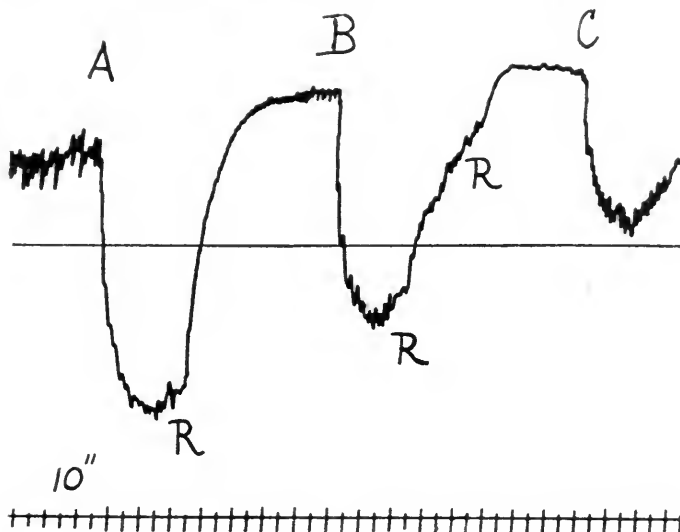


FIG. 13. HORN OF UTERUS OF VIRGIN CAT

Dose in each case 0.002 mgm. adrenaline. At A in Ringer II + 0.05 per cent NaCl (0.95 per cent in all). At B in normal Ringer II (0.9 per cent NaCl). At C in Ringer II diluted with one-seventeenth volume of distilled water (0.85 per cent NaCl).

contraction, a rise in osmotic pressure inhibits existing tonus in isolated plain muscle.

The directly inhibitor effect of a rise in concentration is much better seen in the isolated uterus of the non-pregnant cat, which normally maintains a high tonus in Ringer's solution. Figure 12 shows the inhibitor effect on such a uterus of adding 1 cc. of 25 per cent NaCl to the bath, thus raising the concentration from 0.9 to 1 per cent NaCl. The effect of a small dose of adrenaline is shown for comparison.

This suggests the enquiry, how the effect of an inhibitor drug will be changed by small changes in tonicity of the fluid in which the organ is suspended. The inhibitor effect of adrenaline on the cat's non-pregnant uterus can be repeated indefinitely with fairly uniform result and is, therefore, suited to the experiment. The only difficulty is occasioned by the fact that the inhibitor action produced by increasing concentration persists till the concentration is lowered again. It is not possible, therefore, as in recording the effects of a stimulant drug on the guinea-pig's uterus, to make each effect start from the same tonus-level of the uterine muscle. However, when the changes are within a very limited range, it is easy to demonstrate the adjuvant effect of an increase, and the antagonistic effect of a diminution of tonicity, to the inhibitor action of adrenaline. Figure 13 shows the diminishing effect of equal doses of adrenaline as the concentration of Ringer's solution is lowered from 0.95 to 0.9 and 0.85 per cent NaCl. In this case, again, the effect is not due to specific ionic action; the addition of cane-sugar to the Ringer's solution is as effective in producing inhibition, or augmenting the inhibitor action of adrenaline, as the addition of an osmotically equivalent amount of sodium chloride.

#### SUMMARY AND DISCUSSION OF RESULTS

These experiments, begun with the object of investigating the influence of strong saline in protecting the anaphylactic guinea-pig from the fatal effects of reinjection, have led to conclusions of more general application to the activity of plain muscle. It should be noted that it is with the tonus rather than with the rhythmic activity of plain muscle that the effects described in this paper are concerned; though the probability of their applicability to rhythm also is indicated by a few experiments which I have made on isolated intestinal muscle, of which rhythm is the predominant characteristic, as tone is that of the isolated non-pregnant uterus. The abolition of the rhythm of isolated intestinal muscle, by addition to the Ringer's solution of 1 per cent of dextrose, has been previously described by Cohnheim (11).

But, dealing only with the results described in detail in previous sections, they may be summarised as follows:

1. Any increase in the tonicity of the Ringer's solution, in which isolated plain muscle is suspended, has an inhibitor effect on tonus; any decrease in tonicity acts as a stimulus.

2. When the change in either direction is too small to cause a rise or fall of tonus by itself, or when the plain muscle has adjusted itself to the new conditions, the subsequent effect of a stimulant drug is decreased, that of an inhibitor drug increased, by a rise in tonicity, while a fall in tonicity produces the opposite effects in both cases.

3. The protective effect of strong saline injections in the anaphylactic shock of the guinea-pig is readily explicable, as a particular case of the depressant effect of raised tonicity on the response of plain muscle to stimulant drugs.

It does not seem desirable at the present stage to attempt to base on these results any positive conclusions as to the mechanism of plain muscle contraction. At first sight, indeed, the apparent dependence of the effect on the tonicity of the solution only, and its independence within limits of the nature of the salt or non-electrolyte to which this is due, seems to be inconsistent with theories which attribute contraction to imbibition of water (Quellung) by the muscle-colloids. It must be borne in mind, however, that we are dealing with living cells, freely permeable to water, but relatively impermeable to sodium ions or cane-sugar. The immediate effect of changes in the osmotic pressure of the bathing-fluid, whatever the substance to which the osmotic pressure is due, will be to withdraw water from, or add water to the constituents of the muscle fibers, thereby raising or lowering the concentration of their intrinsic salts. Since the interchange in contraction is regarded as taking place between fibrils and interfibrillar substance, and not between the whole organ and the fluid bathing its surface, there is no necessary inconsistency between the facts here presented and an imbibition theory of contraction. With one particular view of the nature of plain-muscle contraction, on the other hand, they seem to be definitely discordant. This is the theory of Meigs, according to which the

fibers of plain muscle undergo, in contraction, a shrinkage in both dimensions, owing to loss of water to the intervening ground-substance. Meigs (12) bases his theory on histological observations, on which my results have no bearing, and on the behaviour of rings of plain muscle from the frog's stomach extended by a weight, when plunged into distilled water and saturated sodium chloride respectively. In distilled water the ring, after a small preliminary contraction, slowly gives way and lengthens: in saturated salt a small preliminary relaxation, followed by a steady shrinkage, occurs. I would suggest that, if these observations have any bearing on the physiological process of contraction, it is only to the brief initial reaction—the last feeble response of the dying cells—that significance can be attached. The latter portions of Meigs' curves seem to be adequately accounted for by supposing that the dead tissue as a whole shows a diminishing resistance to extension as its cells become disintegrated by distilled water, and that it shrinks as a whole under the dehydrating influence of strong saline. When the changes of tonicity are kept within such limits that they are consistent with the continued vitality of the muscle fibres, as in the experiments with which this paper deals, the effects correspond with the small initial effects mentioned by Meigs, but regarded by him as negligible, in comparison with the secondary and more prolonged effects seen in his experiments.

My object is to place on record observations, made incidentally to another investigation, which may find their place in the development of a theory of plain muscle activity, when further light has been thrown on the subject from other aspects. The results further seem worthy of attention from the purely practical point of view, that they indicate the necessity of precaution against even small alterations in the concentration of the saline bath, when long experiments are conducted on isolated plain-muscular organs, especially when these experiments involve quantitative observations on the degree of response of the plain-muscle to various stimuli. With a shallow layer of warm fluid, and a vigorous stream of oxygen bubbling through it, concentration to a degree sufficient to modify the response in an important degree

might easily occur; and the error would be accentuated, if an effect, in saline thus concentrated, were compared with one produced soon after a change to fluid of the original tonicity.

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## ON THE INFLUENCE OF PHENYLQUINOLIN CARBONIC ACID (ATOPHAN) ON THE URIC ACID ELIMINATION

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Among the various drugs which produce an increased uric acid elimination 2-phenylquinolin-4-carbonic acid appears at the present time to be the most popular, certainly the most extensively discussed in current clinical literature. Judging from the urine analyses accompanying all the communications there seems to be no reason to doubt that the original observations on the subject by Nicolair and Dohrn<sup>1</sup> are correct. All find that the phenylquinolin carbonic acid in question promptly leads to an unmistakable increase in the uric acid output.<sup>2</sup>

Nicolair and Dohrn attributed the increase to a "toxic" production of uric acid, but the later investigators support the view that there is no increase in the total nitrogen output accompanying the increased uric acid elimination and their explanations of the uric acid increase vary according to their point of view concerning the nature of purin metabolism and of gout. Urine analyses alone cannot throw any definite light on the interesting questions involved, and several of the recent investigators, Plehn (l.c.), Retzlaff,<sup>3</sup> Schittenhelm and Ullmann,<sup>4</sup> Wicchowski and Bass,<sup>5</sup> and Zeulzer,<sup>6</sup> have accordingly attempted to determine

<sup>1</sup> Deutsches Archiv f. Klin. Med., lixiii, 331, 1908.

<sup>2</sup> Weintrand: Deutsches Archiv. f. Klin. Med., lixiii, 1908; Dohrn: Zeitschrift f. Klin. Med., nos. 5 and 6, 1912. Plehn: Deutsch. Med. Wochenschrift, no. 3, 1912.

<sup>3</sup> Zeitschrift f. Exp. Path. u. Ther., xii, 307, 1913.

<sup>4</sup> Zeitschrift f. Exp. Path. u. Ther., xii, 360, 1913.

<sup>5</sup> Wiener Klin. Wochenschrift, no. 47, 1912.

<sup>6</sup> Berl. Klin. Wochenschrift, xlv, no. 47, 1911.

by means of blood analyses whether the increase in the uric acid output represents a corresponding decrease of the uric acid in the blood.

The only method as yet available for the study of uric acid in blood including the necessary determinations of the amount in normal blood is the colorimetric method recently described by Folin and Denis.<sup>7</sup> The earlier methods based as they are on the quantitative isolation of pure uric acid from the blood must almost of necessity fail since success or failure in this field depends on finding the last 1 or 2 mgm. of uric acid per 100 grams of blood. Wiechowski and Bass (l.c.) have recently published a method for which they claim the necessary accuracy and they report finding uric acid in normal human blood by their method corresponding to the figures reported by Folin and Denis. But in as much as they also report results which differ from those which we have now obtained with reference to the effect of phenylquinolin carbonic acid on the uric acid content of blood, we are skeptical as to the possibility of isolating in pure condition all the uric acid in blood by the method which they describe.

The experiments described below were undertaken for the purpose of determining whether phenylquinolin carbonic acid does or does not reduce the uric acid content of the blood. The patients selected for the investigations with one exception (No. 2, E. P. McG.) had gout. The latter was included because though kept on low nitrogen diets his blood had been found to contain abnormally high amounts of uric acid. The patients were all kept on an approximately purin free diet for two to three days before taking the first sample of blood.

*Experiment I.* G. W. S., street car conductor, age 48. Much gout and rheumatism, in family. Excessive use of alcohol. First attack seventeen years ago. Several severe attacks since. Chiefly in metatarsal phalangeal joints of both great toes. In hospital for several weeks two years ago with typical attack. Joints normal except those of the great toes which were swollen, red and moderately tender, the toe bent outward and the skin red and shiny.

<sup>7</sup> Journal of Biol. Chem., xiii, 469, 1913.



Day	URINE			BLOOD (100 GRAMS)			
	Volume	Nitrogen	Uric acid	Uric acid	Non-protein nitrogen	Urea nitrogen	Atophan
	cc.	grams	grams	mgm.	mgm.	mgm.	grams
1	1060	7.2	0.29	5.4	36	17	
2	2200	7.9	0.81				5
3	2000	7.4	0.82	3.4	35	17	5

*Experiment II.* E. P. McG. Age 23. Rejected for life insurance on account of albuminuria and cast. No other disease. On low protein diet for several weeks before beginning of experiment. Atophan was given because previous examinations had shown high uric acid content of the blood.

Day	URINE			BLOOD (100 GRAMS)			
	Volume	Nitrogen	Uric acid	Uric acid	Non-protein nitrogen	Urea nitrogen	Atophan
	cc.	grams	grams	mgm.	mgm.	mgm.	grams
1	1000	9.6	0.6	5	23	11	
2	1160	9.4	0.54				3
3	1060	9.4	0.75				3
4	990	10.6	0.78	1.1	28	14	3

*Experiment III.* J. O' G., longshoreman. Age 58. No gout or rheumatism in family. At least ten pints of ale a day besides whiskey. Fourteen years previously had very sudden and severe attack of pain in right metatarsal phalangeal joint which was red "looked as if it were going to burst and so tender that he could not touch it with a feather without pain." Kept him from work for five weeks. Two or three attacks since. Two years previously began to develop cardiac symptoms. Tophi in ears. Toe joints red and somewhat enlarged. Arteriosclerosis.

Day	URINE			BLOOD (100 GRAMS)			
	Volume	Nitrogen	Uric acid	Uric acid	Non-protein nitrogen	Urea nitrogen	Atophan
	cc.	grams	grams	mgm.	mgm.	mgm.	grams
1	1800	14.9	0.52	3.1	32	17	
2	1500	17.2	0.76				5
3	1400	18.2	0.72				5
4	1000	12.8	0.66	1.2	30	16	

*Experiment IV.* D. J. H. No regular employment. Age 36. Knows nothing about family. Six to eight whiskies a day for twenty-five years. Wasserman negative. First attack three months previously. Excessive pain in right hand and elbow which were red, swollen and very tender, extended to both great toe joints. Tophi in both ears which proved to be urates. Joints of the right hand and two middle fingers of left hand enlarged. Both great toe joints enlarged, red and tender, also tenderness over the right sacroiliac joint. X-ray picture of both great toe joints typical for gout. Temperature 100.9° on admission but normal since. Urine, clear, acid, slight trace of albumen, few fine granular casts.

Day	URINE			BLOOD (100 GRAMS)			
	Volume	Nitrogen	Uric acid	Uric acid	Non-protein nitrogen	Urea nitrogen	Atophan
	cc.	grams	grams	mgm.	mgm.	mgm.	grams
1	990	7.8	0.22	5.7	60	32	
2	775	6.5	0.42				5
3	1500	8	0.44				
4	1710	10.6	0.35	2.2	32	17	

*Experiment V.* D. N., waiter, negro. Age 58. No known gout in family. History of probable lues. Alcohol not excessive. Four months previously had severe pain in the dorsum of the right foot. Pain in foot and great toe joint ever since which has at times been violent. Temperature normal. Urine, cloudy, acid, slight trace of albumen, rare hyaline and granular casts. X-ray shows typical gout in hands and feet. Right metatarsal phalangeal joint of big toe very tender. Not red or swollen. No other tenderness. Arteriosclerosis and hypertension.

Day	URINE			BLOOD (100 GRAMS)			
	Volume	Nitrogen	Uric acid	Uric acid	Non-protein nitrogen	Urea nitrogen	Atophan
	cc.	grams	grams	mgm.	mgm.	mgm.	grams
1	1600	10.2	0.22	3.1	44	21	
2	1100	8.3	0.53				5
3	1450	10.5	0.50				5
4	1190	9.6	0.45	2.2	36	18	

So far as the uric acid in the urine and blood is concerned the results recorded in the above five experiments appear to us to be quite unmistakable. The administration of the phenylquinolin carbonic acid in every case led to an increase in the uric acid elimination and to a marked reduction of the uric acid in the blood. The increased output therefore represents the elimination of uric acid which had previously accumulated in the blood and the previous accumulation represents a corresponding kidney inefficiency.

By means of non protein nitrogen and uric acid determinations in blood Folin and Denis<sup>3</sup> have recently obtained data which show that the retention of uric acid in the blood is not always accompanied by a retention of non protein nitrogen and urea, and in other cases they found retention of nitrogen unaccompanied by an accumulation of uric acid. In the analyses recorded above we have encountered the same condition. In the first three experiments the non protein nitrogen is either normal or (experiment I) but little above the normal, notwithstanding the high uric acid figures. In the last two experiments, on the other hand, the non protein nitrogen in the blood accompanying the uric acid is very high, as high as in many cases of chronic nephritis.

In the absence of any other reasonable explanation such independent variations in the accumulation of different products in the blood must be explained on the basis of a selective activity on the part of the kidney and corresponding to such a selective activity a corresponding partial and selective deterioration. The interesting fact to be noted in this connection is that the phenylquinolin carbonic acid not only brings about a diminution of the uric acid in blood but also seems to lead to a diminution of the non protein nitrogen and urea whenever these are present in the blood in unusual amounts (experiments IV and V). Weintraud<sup>4</sup> has already recorded some observations on the effect of atophan

<sup>3</sup> Journal of Biol. Chem., xiv, 29, 1913.

<sup>4</sup> Therapeutische Monatshefte, January, 1912.

on the "rest" nitrogen of the blood (in nephritis) and has found a considerable reduction.<sup>10</sup>

In addition to the experiments recorded above we wish to record some observations on one particularly severe case of gout which in some respects is quite remarkable.

*Experiment VI.* J. G. K., butcher. Age 55. No gout in family. Moderate alcohol but a heavy eater. Wasserman negative. First attack fifteen years previously in left index finger. Attacks since then in both ankles, both knees, both great toe joints and in right hand. Temperature 102° on entrance to the hospital. Urine clear, acid, slight trace of albumen, few fine granular casts. X-ray typical of gout. Large, obese man. Large tophi over both elbows, both patellae and right tendo achilles. Right hand and left index finger red, swollen and tender. Metatarsal phalangeal joints of both great toes much enlarged.

From a large abscess on the forearm we obtained by aspiration about 15 cc. pus which on microscopic examination showed an abundance of urate needles. A portion of the fluid, dried at 105°C. for forty-eight hours, was assayed colorimetrically and found to contain 20 per cent uric acid calculated on the dry weight.

URINE				BLOOD (100 GRAMS)			
Day	Volume	Nitrogen	Uric acid	Uric acid	Non-protein nitrogen	Urea nitrogen	Atophan
	cc.	grams	grams	mgm.	mgm.	mgm.	grams
1	1000	11.2	0.61	2.4	35	18	
2	940	12.7	0.56				5
3	1100	12.6	0.64				
4	1300	16.1	0.64	2.1	42	22	

In this case the administration of the phenylquinolin carbonic acid brought about no relief to the patient. The uric acid elimination was rather high throughout and the drug did not increase the uric acid output nor did it bring about an appreciable dimin-

<sup>10</sup> Systematic studies of the whole subject of retention of waste nitrogen by the help of our new analytical methods have been in progress for several months in this laboratory, and we intend to include in this work the study of the effect of atophan on the retention.—Folin.

ution of the uric acid in the blood. But the initial uric acid content of the blood was practically normal.

The figures recorded in the last table represent a curious exception to the rule that the blood of those suffering from gout is invariably high in uric acid. Because of the unmistakable abundance of urate deposits in this patient we continued to make examinations for uric acid and in about two weeks the uric acid in the blood began to increase. Accordingly we repeated the administration of atophan and this time it was accompanied by the usual increase of the uric acid in the urine and a diminution of the same in the blood.

*Experiment VI—continued*

Day	URINE			BLOOD (100 GRAMS)			Atophan
	Volume	Nitrogen	Uric acid	Uric acid	Non-protein nitrogen	Urea nitrogen	
	cc.	grams	grams	mgm.	mgm.	mgm.	
1	1400	18.2	0.88	3.6	35	19	
2	840	11.1	0.50				
3	990	11.2	0.42				
4	1110	12.6	0.43				
5	1000	11.4	0.39				
6	1060	12.7	0.39	5.1	42	23	3
7	810	8.6	0.66				3
8	1000	10.5	0.82				3
9	820	13.3	0.76				3
10				1.6			3

The results obtained from this patient furnish a striking illustration of the fact that phenylquinolin carbonic acid acts on the kidneys (Weintraud) and does not "mobilize" (Brugsch)<sup>11</sup> deposited urates. The variations in the uric acid output and in the uric acid retention appears to be chiefly or wholly due to variations in the activity of the kidneys. In the first part of experiment VI substantially normal amounts of uric acid were eliminated and the blood was normal; in the later period the

<sup>11</sup> Berliner Klin. Wochenschrift, xl, no. 34, 1912.

uric acid elimination was much smaller and the uric acid accumulated in the blood until we administered the atophan.

Finally we give the figures obtained from one of us (H. L.) who is in good health and never has had an attack of gout but in whose family there has been more or less of this disease.

Day	URINE			BLOOD (100 GRAMS)			
	Volume	Nitrogen	Uric acid	Uric acid	Non-protein nitrogen	Urea nitrogen	Atophan
	<i>cc.</i>	<i>grams</i>	<i>grams</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>grams</i>
1	1440	12.6	0.66	4.0	29	13	
2	2000	12.6	1.17				3
3	1600	11.2	0.87				3
4	1800	10.9	0.86				3
5				1.5	25	13	

We are indebted to Dr. Locke of the Boston City Hospital and to Dr. Edsall of the Massachusetts General Hospital for turning over to us the subjects of this investigation together with the clinical histories. The latter have been given in abbreviated form since this is not a clinical paper.

# THE ACTION OF SO-CALLED EMMENAGOGUE OILS ON THE ISOLATED UTERINE STRIP

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In August, 1912, I have had the opportunity to investigate a case of pennyroyal poisoning, which I have reported elsewhere.<sup>1</sup> Pennyroyal (oleum hedeomae and oleum pulegii) is but one of a number of drugs which have for a long time enjoyed the popular reputation of being efficient emmenagogues and abortifacients; and some of these are still official in the United States, the British, and other pharmacopoeias. Although the pharmacological action of some of these, notably pennyroyal,<sup>2</sup> and the French preparation *apiol*,<sup>3</sup> has been experimentally studied, none of them have been investigated in respect to their specific action on the uterus,<sup>4</sup>—the purpose for which they are mostly employed.

I have therefore undertaken to study the effect of these substances upon the isolated uterus, to determine if possible the mode of their "emmenagogue" or abortifacient action.

## METHOD

The horns of a cat's uterus, pregnant and virgin, were employed, and the apparatus used was very much like that used by Dale and Laidlaw in the work on standardization of pituitary extracts.<sup>5</sup> The uterine strip from a freshly exsanguinated animal is suspended in a small glass chamber, one end being fixed to the bottom of the chamber, the other or free end being attached

<sup>1</sup> Jour. Am. Med. Assn., July 12, 1913.

<sup>2</sup> Lindeman: Archiv für Exp. Path. 4. Pharmak, xlii, p. 356.

<sup>3</sup> Hefter: Archiv für Exp. Path. 4. Pharmak, xxxv, p. 365.

<sup>4</sup> Meyer und Gottlieb: Experimentelle Pharmakologie, 24 ed., p. 202.

<sup>5</sup> Dale and Laidlaw: Jour. of Pharm. and Exp. Therap., iv, p. 75.

to the short arm of a long and lightly weighted lever. The chamber is filled with Locke's solution kept at a very constant temperature, 38°C. through which a constant stream of oxygen is kept bubbling. The bottom of the chamber is drawn out into a tube to which a piece of rubber tubing is attached and clamped off *at will*, for the purpose of drawing off and changing the solution whenever desired. The whole apparatus is immersed in a water-bath or jacket, for the regulation of the temperature. Extreme care must be taken to keep the temperature and inflow of oxygen constant, as otherwise the curves obtained show variations due to these factors. Furthermore the Locke's solution should be very carefully prepared, using *glass-distilled* water, as ordinarily distilled water contains enough extraneous salts to affect the sensitiveness of the preparation.

The manipulation and exposure of the uterus, followed by immersion in the warm solution almost invariably produce a high degree of tonus in the suspended strip. This tonus, however, slowly gives way, with small rhythmic interruptions until a condition of almost complete relaxation is produced. This condition of uniformly low tonus is attained after suspension from fifteen to thirty minutes, when the preparation is ready for testing. The uterus is now either quiescent, or in ideal preparations for our purpose, shows small rhythmic contractions. These contractions are recorded on a kymograph, and after taking a normal tracing, the drug to be tested is introduced.

#### RESULTS

The following oils were studied: *oleum hedeomae* (pennyroyal), *oleum sabinae* (savine), *oleum tanacetii* (tansy), *oleum rutaе* (rue), *oleum thymi* (thyme), *oleum terebinthinae* (turpentine), and apiol.

Weak solutions or emulsions of these were made up with Locke's solution. Controls were also made with an emulsion of a neutral oil (olive oil).

The results obtained may be seen from the figures, all of the substances used, even in small amounts, exhibited absolutely no stimulating action on the uterus. On the contrary they caused it to relax, and even paralyzed it. Thus in figure 1, we note



the immediate inhibition of the contractions of a virgin cat's uterus produced by a small dose of pennyroyal. Figures 2 and 3 show the similar effect of tansy and apiol. A similar action was noted in the case of all the substances studied, the various drugs differing only in the degree of their toxicity. It was found that pennyroyal, tansy, and apiol were the most toxic of the group. As might be expected, the least deleterious of the oils studied was turpentine. It was necessary to use stronger emulsions of this drug to obtain the same effect.



FIG. 1. ACTION OF PENNYROYAL ON THE VIRGIN UTERUS OF CAT

The paralytic action of the oils studied is exerted not only upon the normal uterus, but also upon one which is under the influence of a uterine stimulant, as is shown in figure 4. Here we see the contractions of a uterine strip stimulated by the recently discovered cardiac stimulant, Bufagin,<sup>6</sup> and in turn inhibited by oil of pennyroyal. At *A* we note the increased contractions following a small dose of Bufagin (0.00001 gram; at *B*, the enormous stimulation of contractions and heightened tonus after a larger dose (0.0002 gram), and at *C*, the immediate relaxation caused by the addition of some pennyroyal (5 cc. of 2 per cent solution).

<sup>6</sup> Abel and Macht: Jour. of Pharm. and Exp. Therap., iii, no. 3.

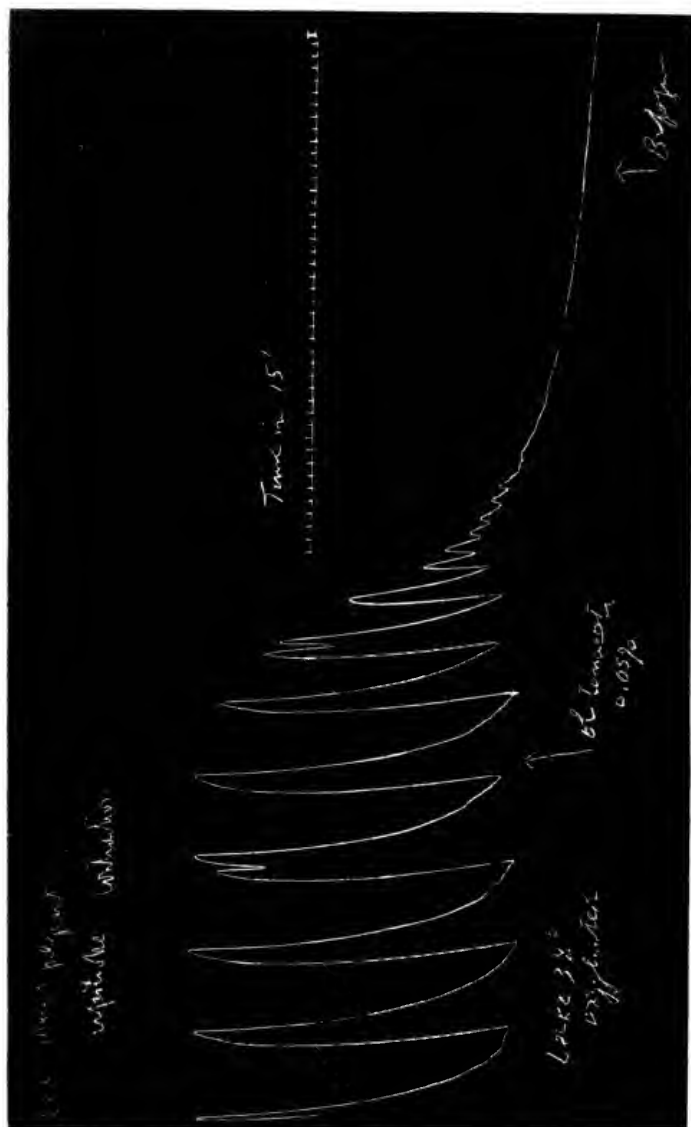


FIG. 2. PREGNANT UTERUS. OIL OF TANSY. BUPAGIN DOES NOT RESUSCITATE



FIG. 3. EFFECT OF APIOLE ON PREGNANT UTERUS

## CONCLUSIONS

From the above observations we are led to conclude:

1. That the so-called emmenagogue oils have absolutely no specific or directly stimulating action on the uterine muscle.
2. That on the contrary they inhibit the contractions of the uterus and even paralyze it.
3. That their "emmenagogue" and abortifacient action is therefore due to general constitutional poisoning or gastro-intestinal irritation and not to any specific action on the uterus itself.

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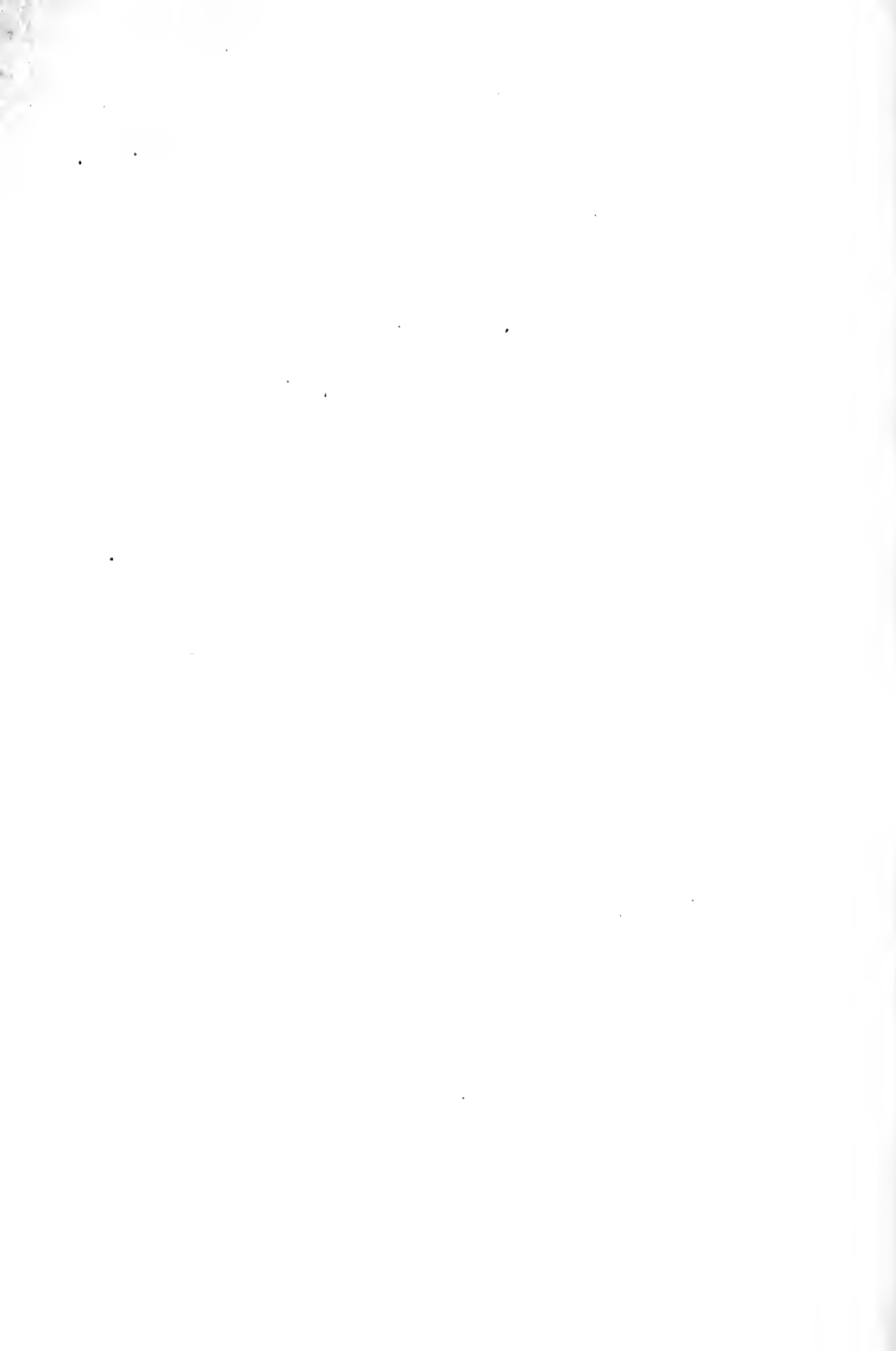
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